2018

Evaluation of Microbially-converted Soybean Meal as an Alternative to Fishmeal in Weaned Pig Diets

Sue Sinn
South Dakota State University

EVALUATION OF MICROBIALLY-CONVERTED SOYBEAN MEAL AS AN ALTERNATIVE TO FISHMEAL IN WEANED PIG DIETS

BY

SUE SINN

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Animal Science

South Dakota State University

2018

EVALUATION OF MICROBIALLY-CONVERTED SOYBEAN MEAL AS AN ALTERNATIVE TO FISHMEAL IN WEANED PIG DIETS

SUE SINN

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Crystal L. Levesque, Ph.D.

Thesis Advisor Date

Joseph Cassady, Ph.D.

Head, Animal Science Date

Dean, Graduate School Date

ACKNOWLEDGEMENTS

I am incredibly thankful to many people for the completion of this thesis. First and foremost, I would like to sincerely thank Dr. Crystal L. Levesque for giving an inexperienced pig handler from Texas the opportunity of a lifetime and for maintaining confidence in me throughout this long but fruitful journey. I would not be anywhere near the agricultural industry without her, and I'm truly grateful.

I would like to thank the rest of my committee members Drs. Bob Thaler, Bill Gibbons, Russ Daly, and Christopher Saunders for the investment of their time, patience, and constructive comments. I would like to thank my fellow graduate and undergraduate students that have helped me in data collection throughout several of my trials including Agatha Ampaire, Ethan Blom, Amanda Cross, Maverick Fiene, Jesse Fulton, Eric Jolitz, Kaleigh Kerns, Jake Koepke, Kara Marley, Cody Milbrath, Stephanie Perkins, Adolf Saskey. A special thank you to Deon Simon, Jason Griffin, and Dr. Jeffrey Clapper for providing me help and advice in the laboratories from the titanium dioxide analyses and bomb calorimeter to the PCR work on gene expression. I would like to thank the barn manager, Amos Wipf, for help on sourcing the pigs for my trials and data collection.

Support for this research was provided by the South Dakota Soybean Research and Promotion Council, National Pork Board, United Soybean Board, South Dakota Game, Fish and Parks, South Dakota State University Department of Natural Resource Management, and South Dakota Agricultural Experiment Station. Fish handling and sampling procedures were conducted in compliance with the Animal Welfare Act (South

Dakota State University Animal Care and Use Committee protocol approvals #11-62A and 12-101A).

Lastly, I would like to thank my loving family that maintained unwavering support throughout.

TABLE OF CONTENTS

ABBREVIATIONS

AA	amino acid(s)
AID	apparent ileal digestibility
ANF	anti-nutritional factor(s)
ADG	average daily gain
BW	body weight
CD	crypt depth
d	day
ETEC	enterotoxigenic *Escherichia coli*
F/G	feed : gain
FM	fishmeal
g	gram
G/F	gain : feed
GIT	gastrointestinal tract
h	hour
H&E	Haematoxylin and Eosin stain
IU/g	international unit/gram
kg	kilogram

LT	heat-labile enterotoxin
MCSBM	microbially-converted soybean meal
min	minute
PAS	Periodic acid-Schiff stain
PWDS	post-weaning diarrhea syndrome
SBM	soybean meal
ADRDL	South Dakota Animal Disease Research & Diagnostic Laboratory
SDSU	South Dakota State University
SE	Standard error
SID	standardized ileal digestibility
ST	heat-stabile peptide toxin
U/g	Units/gram
µm	micrometer
VFD	veterinary feed directive
VH	villous height
VH:CD	villous height: crypt depth
wk	week
yr	year

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

EVALUATION OF MICROBIALLY-CONVERTED SOYBEAN MEAL AS AN ALTERNATIVE TO FISHMEAL IN WEANED PIG DIETS

SUE SINN

2018

Digestibility values determined in growing pigs may not apply to nursery pigs; thus, standardized ileal digestibility (SID) of AA in MCSBM and fishmeal (FM) were determined using 30 ± 1.6 kg BW ileal-cannulated barrows (n = 6) and 9.8 ± 1.2 kg BW barrows (n = 37; serial slaughter). Experimental diets included MCSBM, FM, and nitrogen-free where FM and MCSBM were included as the sole protein source. The SID of AA was 3-5% lower in MCSBM than FM when fed to 30 kg pigs. The SID of Arg and Met was greater ($P < 0.05$) in MCSBM than FM when fed to 10 kg pigs. The SID of AA was 12-20% lower in FM when fed to 10 versus 30 kg pigs but only 3-9% lower in MCSBM. A total of 336 barrows and gilts were weaned at 21 d of age (initial BW 6.1 ± 0.8 kg) and used in a performance trial. Pens of pigs were assigned to one of 6 experimental diets (8 pens/diet in two blocks). Treatment diets were fed in Phase I (7 d) and Phase II (14 d) with all pigs fed a common Phase III diet (14 d). Experimental diets included: 1) negative control (NEG) containing corn, soybean meal and whey, 2) NEG + acidifier (NEGA), 3) NEG + FM (POS), 4) POS + acidifier (POSA), 5) NEG + MCSBM (MCSBM), and 6) MCSBM + acidifier (MCSBMA). The FM and MCSBM were included at 7.5% and 5.0% in Phase I and II diets, respectively. Diets were formulated to meet the standard nutrient requirements for weaned pigs. Pig BW and feed disappearance

was measured weekly and fecal scores were measured daily for the first 14 d post-weaning as an indicator of PWDS. Performance (BW, ADG, ADFI, and G/F) was not significantly different among treatments. Treatment for PWDS occurred on different days in each block. Analysis of fecal score was completed separately by block. Pigs fed the NEG diets had higher (P = 0.02) fecal scores than pigs fed the POS diets on d 2 and 3 (block 1) and higher (P < 0.05) than pigs fed MCSBM or POS diets and diets with dietary acidifier on d 6 and 3 (block 2). At the end of Phase I and II, one pig/pen was humanely euthanized for digesta and tissue collection. Digesta pH was measured in the pyloric region of the stomach, duodenum, middle jejunum, ileum, cecum, and middle colon. There was an effect of location ($P < 0.0001$), where the pH was lowest in the stomach and increased until the ileum with a slight dip in the cecum and increased in the colon. There was no effect of ingredient, dietary acid, or their interaction within the gastrointestinal tract. At the end of Phase I, pigs fed NEG and POSA diets had similar pH ($P > 0.10$) from the stomach to the duodenum, and pigs fed NEG, NEGA, and POSA diets had lower ($P < 0.05$) pH from the duodenum to the jejunum. At the end of Phase II, pigs fed NEGA diets had similar ($P > 0.10$) pH from the stomach to the duodenum, and pigs fed MCSBM diets had lower ($P < 0.05$) pH from the duodenum to the jejunum. There was no effect of ingredient, dietary acidifier, or their interaction in villus height, crypt depth, villus height:crypt depth, goblet cell area, Ki-67, inflammation scores in the stomach and duodenum, and mucin scoring in the stomach and upper duodenum at the end of Phase I. Phase II samples were not measured for gut health and function (other than pH measurements). Based off the lack of differences in growth performance and gut

health measurements, MCSBM holds promise as an alternative for FM in nursery pig diets.

Key Words: digestibility, gut health, performance, protein sources, weaned pigs

1.0 LITERATURE REVIEW

1.1 Introduction

Conventional weaning, especially young wean ages, is one of the costliest obstacles in the U.S. pig industry. Reduced performance is commonly seen in the initial weeks following weaning as pigs are introduced to many stressors in combination with a developing digestive system. Wean age plays a role as a potential stressor in the conventional weaning system and it was reported pigs weaned even at 14 d of age gained less than a nursing pig (Leibbrandt et al. 1975). When pigs were weaned at 21 d, income over cost/pig increased by nearly $3 US/head compared to 18 d and more than doubled compared to pigs weaned at less than 15 d of age (Main et al., 2005). In general, the younger the pig at weaning, the less developed digestive tract it will have, which increases the potential for reduced performance and post-weaning diarrhea syndrome (PWDS). Nutrition has played a major role in combatting the reduced performance and PWDS, which includes utilizing different high-protein quality ingredients, such as fishmeal (FM), high-protein plant feedstuffs, such as soybean meal (SBM) or further processed SBM, easily digestible ingredients (such as whey), feed additives (such as dietary acidifiers), and feed grade medication. As the pig ages, the digestibility of fat and protein increases via increased enzyme activity (Leibbrandt et al., 1975) and the need for the highly digestible starter diets reduce.

1.2 Weaning

Natural weaning has been described as a process that “involves the progressive reduction in the rate of milk transfer from mother to young, accompanied by an

increasing intake of solid food by the offspring and profound behavioural changes in the parent offspring relationship" (Martin, 1984). With pigs, natural weaning begins as early as wk 2 when the litter leaves the nest area to follow the sow and mingle with the rest of the herd (Stangel and Jensen, 1991) and is completed between wk 11 and 18 of lactation (Newberry and Wood-Gush, 1985). However, in conventional pork production systems, pigs are generally weaned by abrupt maternal separation at 17 to 28 d of age, and in the early weaning system, pigs are weaned at 12 to 14 d of age (Ko et al., 2015). In the conventional system, the young animals are moved to new housing conditions and mixed with unfamiliar peers (Johnson and Marchant-Forde, 2009) and thus piglets are still socially and nutritionally dependent on the dam (Newberry and Swanson, 2008; Weary et al., 2008). Under natural weaning conditions, piglets will gradually reduce milk intake, increase solid food intake, and increase social independence from the dam (Weary et al., 2008). Within the conventional system, the new physical and social stressors, including separation from the dam and litter-mates, moving to a new pen, and mixing with unfamiliar pen-mates can cause a high amount of distressed vocalization and activity, such as aggression or escape behavior. A slow growth rate immediately after weaning is commonly seen in the pork industry and leads to economic losses (Weary et al., 2008). Minimal feed and water intake immediately post-weaning, including a change in diet, may lead to gut inflammation and pathogenic bacterial colonization, which contribute to PWDS.

1.2.1 Reduced performance

Within the conventional weaning process, growth performance is delayed in the immediate post-weaning period because feed intake is typically minimal (Leibbrandt et

al., 1975). The changes in social structure and environment with weaning is one of the causes of the reduced feed intake as the pigs must adapt to new pen-mates and environment. More time may be spent in aggressive, vocalization, and escape behavior instead of exploration for feed. However, the extent and duration of this low or underfeeding period can be highly variable among pen-mates where some pigs find and consume their first feed within 3 min and other pen-mates not until 54 h after weaning (Brooks et al., 2001). Due to the minimal feed intake, the metabolizable energy intake is often insufficient to meet the pig's requirement for gain or even to maintain BW for the first 3 to 5 d post-weaning. As a result, pigs may depend on body energy reserves to maintain growth (van Beers-Schreurs and Bruininx, 2002). The impact of a poor start to the nursery can also predispose the pig to future growth loss potential as the early performance delay may negatively affect body thermal regulation and gastrointestinal morphology and physiology (Brooks et al., 2001; Hötzel et al., 2011). Pigs that gained greater than 0.23 kg/d during the first wk post-weaning was approximately 7.71 kg heavier at slaughter compared to pigs that lost weight during the first wk post-weaning (Kats et al., 1992). Wean age and diet complexity may also play a role in immediate or subsequent performance. Pigs had similar body weight (BW) regardless of wean age at wk 6 of age but concluded pigs weaned at a greater BW may adapt to the post-weaning environment more quickly than pigs weaned at a lower BW (Leibbrandt et al., 1975). Younger pigs had a lower BW at finishing compared to older pigs by approximately 6.5 kg (Davis et al., 2006). On the contrary, in another study, wean age did not affect age at 109 kg (Dritz et al., 1996). In that same study, diet complexity did influence age at 109

kg with pigs on a low-complexity diet were older than pigs on a medium-complexity diet (Dritz et al., 1996).

The diet change from liquid milk provided by the sow to solid dry feed is a stressful event for pigs which also contributes to the minimal feed intake (van Beers-Schreurs and Bruininx, 2002). When pigs are weaned at the U.S. industry standard of 3 wk of age, they have had little if any exposure to dry feed (Le Dividich and Sève, 2000) as opposed to the potential to forage with the herd in natural weaning. The concept of liquid feeding compared to dry feeding was previously tested as a means to combat the voluntary reduced feed intake in the immediate post-weaning period. Pigs offered liquid feed or fermented liquid feed had greater feed intake compared to dry feeding (Brooks et al., 2001). However, there were problems associated with liquid feeding that made it impractical in practice. If a liquid feeding system is used, the producers must increase the labor involved in replenishing feeders and cleaning and the microbiological load in the feed needs to be maintained (Brooks et al., 2001). The underfeeding period and reduced growth performance is unavoidable in practice, whether the pigs are abruptly weaned from liquid to solid feed or have the potential digestive disturbances caused by an intense liquid feeding program (Le Dividich and Sève, 2000). Feeding liquid milk can be associated with the development of acute diarrhea due to ETEC (Brooks et al., 2001). Therefore, other substitutions would have to be considered such as a complex starter diet, dietary acidifiers, and antibiotics to reduce consequences from weaning and stimulate feed intake in the immediate post-weaning period.

Along with minimal feed intake, water intake may also be a factor that leads to reduced performance throughout the growing period for pigs. Dehydration can be a

concern in the immediate post-weaning period due to stress-induced lethargy or lack of access to water during transit and may have more physiologically immediate and severe consequences than reduced feed intake (Horn et al., 2014). It may take longer than 7 d for a pig to restore its daily fluid intake to be equivalent of what it was the day before weaning (van Beers-Schreurs and Bruininx, 2002). There was an 80% reduction in average daily gain (ADG) in pigs restricted from water for 24 h compared to pigs that were not restricted from water for 1 d immediately after weaning (Horn et al., 2014). The group also reported that pigs experienced compensatory gain within the first 7 d after being restricted from water for 24 h and had an improved feed efficiency compared to the pigs that were not restricted from water at all (Horn et al., 2014).

Abrupt weaning is one of the most stressful events for a pig in today's production system. With the introduction of solid feed and absence of sow milk, change in environment and pen-mates, a reduction in growth performance is commonly observed in commercial nurseries along with the development of PWDS.

1.2.2 Digestive development of the weaned pig and post-weaning diarrhea syndrome

The stomach has a number of major functions in digestion which include storing, mixing, and partially digesting food (Zhang and Xu, 2006), and as the pig ages, volume and acid secretion will increase (Manners, 1976). The major changes in the stomach that occur from suckling through the immediate post-weaning phase relate to changes in pH, development of gastric acid, and enzyme secretions (Heo et al., 2013). The optimal pH level for dietary protein digestion is 3.0 (Heo et al., 2013). The stomach of a young pig cannot be maintained at a low pH; therefore, the stomach pH is generally around the same pH as the diet (Manners, 1976). Weaned pigs have been reported to have a greater

gastric pH compared to suckling pigs at the same age, but this may be due to greater feed intake coupled with the inability to secrete enough acid for the stomach content and a possible difference in buffering capacity with dry feed in comparison to sow milk (Efird et al., 1982). In addition to a more efficient protein digestion, a lower pH (pH of 3 to 4) can help aid in reducing enteric disease (i.e. PWDS) due to the bactericidal environment and prevention of pathogenic bacteria, such as *E. coli*, passing into the small intestine (Heo et al., 2013). The weaned pig may also experience gastric stasis or reduced rate of gastric emptying (Heo et al., 2013); dietary characteristics can attribute to gastric stasis with pigs on pelleted, dry diets having 2 to 5 times longer gastric emptying rate compared to suckling pigs (Cranwell, 1985). Reduced gastric emptying, coupled with a greater pH, allows for a more conducive environment for the proliferation of pathogenic bacteria, assisting in the development of PWDS (Heo et al., 2013). The pH in the stomach will begin to lower to the ideal acidity level when the pig is approximately 7 wk of age or older with the increased secretion of hydrochloric acid (Manners, 1976). Dietary protein digestion begins in the stomach with hydrochloric acid-activated pepsin (Khan et al., 1999). The production and activity of digestive enzymes observed around weaning are affected by many factors, including stress, rate and amount of feed consumption, and the diet (Hedemann and Jensen, 2004). Pepsin activity to be the lowest (1309 U/g of tissue) in the fundic region of the stomach 2 d post-weaning but increased by 5 d post-weaning (Hedemann and Jensen, 2004). Alternatively, in another study, pepsin activity increased after weaning (Cranwell, 1985). In a different study, pigs were weaned at 4 wk of age and were fed either a corn and SBM based diet or corn, SBM, and whey based diet; gastric

proteolytic enzymes increased 1 wk post-wean, but the chymotrypsin and trypsin activity in the pancreas decreased 1 wk post-wean (Lindemann et al., 1986).

The small intestine is comprised of the duodenum, jejunum, and ileum, and the main purpose of the small intestine is to continue the digestion process from the stomach, prepare digesta for absorption, and absorb the nutrients (Zhang and Xu, 2006). Although the immediate post-weaning period is characterized by poor growth performance with low feed intake, early weaned pigs had greater intestinal weights per kg of empty body compared to suckling pigs at the same age (Cera et al., 1988). The same study concluded a higher percentage of nutrients are used for intestinal tissue development after weaning (Cera et al., 1988). In general, there are a few factors that affect the alterations in gut morphology after weaning and may contribute to scouring, including but not limited to: maladaptation to the physical and psychological weaning stressors, the withdrawal of sow's milk and dietary change, and enteropathogenic bacteria and their interactions in the small intestine (Pluske et al., 1997).

Within in the mucosal layer of the small intestine are villi, which are finger-like projections that increases the luminal surface area and aid in the digestive and absorptive processes (Zhang and Xu, 2006). Villous atrophy can be caused by either an increased rate in crypt cell production (i.e. microbial challenge or antigenic compounds from feedstuffs) or a reduced rate of cell renewal from fasting (Pluske et al., 1997). Reduced feed consumption after weaning may lead to sup-optimal protein and energy intakes, which may reduce crypt-cell production rate, and thus, be a cause to villous atrophy due to the slowing of new crypt cell production (Hall and Byrne, 1989). If the pig experiences a feed fasting for 24 h, the villi in the small intestine can be shortened and stunted. The

shortened villi of the small intestine will typically be at its lowest cell growth or regeneration 5 d post-weaning, especially in the lower ileum and upper jejunum (McCracken and Kelly, 1993). Reductions in villous height (VH) throughout the small intestine by 20 to 35% in 21 d old weaned pigs (Hampson, 1986). At 25% of the length along the small intestine, VH values were less than 700 µm for weaned pigs, while suckling pigs had VH values greater than 850 µm at 22 d of age (Hampson, 1986). The VH continued to reduce for 5 d post-weaning until it was nearly half of what it was at weaning. Similarly, there was a dramatic decline in jejunal VH, regardless of wean age (21 or 35 d), at 3 d post-wean but an increase in VH with age (Cera et al., 1988). In comparison, 21 d old suckling pigs had little alterations in VH throughout the small intestine (Hampson, 1986) Crypt elongation occurred normally in unweaned pigs but is increased with immediate post-weaned pigs and the VH began to increase 5 to 8 d after weaning (Cera et al., 1988).

Measuring brush border enzyme activities are parameters of intestinal function in the post-weaned pig. The developing function of the small intestine can be a contributing factor to the reduced growth rate immediately post-wean with reduced brush border enzyme activities which can increase malabsorption from the small intestine of the young pigs (Hampson and Kidder, 1986). The reduction in the villous height: crypt depth (VH:CD) are typically associated with the reduction in brush border enzyme activities, such as sucrase and lactase. When weaned at 21 d of age, there was a large, continuous decline in lactase activity along the small intestine from 171 IU/g of mucoal protein to 46 IU/g of protein 11 d post-weaning (Hampson and Kidder, 1986). Regardless of age in that same study, lactase activity is greatest at 21 d and will decrease as the pig ages (Hampson

and Kidder, 1986). Sucrase activity initially declined to minimal values to approximately 20 IU/g of mucosal protein 4 to 5 d post-wean and recovered by 11 d post-wean to 31 IU/g of protein. Brush border enzyme secretions may be influenced by the practice of creep feeding. Creep feed is a highly palatable and digestible diet that can be provided to the litter prior to weaning (Bruininx et al., 2002). Providing a litter with creep feed may combat the "malabsorption syndrome" immediately post-weaning; however, it is suggested litters must consume 600 g of creep feed for this effect to be evident in piglet growth. Pigs consumed little amounts of creed feed until 18 d of age with an average of 236 g per litter (Hampson and Kidder, 1986). The insufficient feed intake to meet nutrient requirements in the immediate post-wean period can lead to an impaired intestinal tract, such as villous atrophy, increased crypt depth (CD), and opportunity for the proliferation of *β*-haemolytic enterotoxigenic *Escherichia coli* (ETEC) in the lower intestinal tract (van der Meulen et al., 2010).

Post-weaning diarrhea is a condition commonly observed in the first 2 wk where pigs will have frequent discharge of watery feces and is typically associated with a large number of ETEC (such as adhesins K88 and F18) shed through the feces (Heo et al., 2013); however, the main cause of PWDS is the weaning itself, usually because the pigs are weaned at 3-4 wk of age (Nagy and Fekete 1998). For the ETEC strains, fimbriae attach to the glycoprotein receptors of the microvillous enterocytes within the brush borders of the small intestine (Nagy and Fekete, 1998). With adhesion comes colonization of the gastrointestinal tract where the ETEC pathogens release enterotoxins. Enterotoxins are extracellular proteins or peptides, which can exert their actions on the intestinal epithelium (Nagy and Fekete, 1998). ETEC strains are characterized by the

production of either enterotoxin categories: LT which have a large molecular weight or ST which have a small molecular weight and can resist up to 100°C for at least 15 min (Nagy and Fekete, 1998). As for ETEC associated with PWDS, the most common enterotoxins are either heat-labile enterotoxin (LT), STa, or STb (Nagy and Fekete, 1998). The LT toxins increase the secretion of sodium, chloride, and hydrogen carbonate ions into the lumen, while the heat-stabile peptide toxins (ST) reduce the absorption of liquids and salts. With secretion of either of the toxins, the outcome will be a hypersecretion of water and electrolytes into the small intestinal lumen, exceeding the absorptive capacity of the colon (Nagy and Fekete, 1998). The LT and STa enterotoxins induce a secretory diarrhea without epithelial damage, but STb involves shortening of the small intestinal villi and immediately after weaning pigs become more susceptible to the ST enterotoxins (Nagy and Fekete, 1998). It is important to understand there may also be other *E. coli*, not enterotoxigenic, involved in PWDS with different types of bacterial fimbrial adhesins that attach to the intestinal mucosa (Heo et al., 2013).

There are a few innate defense mechanisms that protects the weaned pig from the colonization of pathogens, such as ETEC. Secretory mucins are secreted from goblet cells, which makes up mucin layers along the epithelial layer of the gastrointestinal tract and provide a physical barrier. The mucin can be classified as neutral or acidic subtypes; acidic mucin is predominantly in the intestinal tract and is thought to provide protection against bacterial translocation (Deplancke and Gaskins, 2001). In addition to the mucosal defense, the commensal microbiota of the intestinal tract provides a large influence on overall health by facilitating nutrient uptake, stimulating the immune response, and protecting the host from pathogenic bacteria (Konstantinov et al., 2006). During the

birthing process and immediately afterwards, the gastrointestinal tract of the neonate will be colonized from the mother and surrounding environment, which leads to a dense and complex development of microbiota (Mackie et al., 1999). As soon as the pig is weaned, the piglet stops receiving antibodies from the sow milk (van Beers-Schreurs and Bruininx, 2002). It is believed pigs in the immediate post-weaning period are likely to experience profuse, watery diarrhea from proliferation of pathogenic bacteria, such as ETEC, but this may also be caused by an accumulation of lactate or succinate in the intestine along with the insufficient development of intestinal microbiota and mucosal immune system (Konstantinov et al., 2006). Treatment for PWDS typically includes oral antibiotics and fluid electrolyte replacement, while good farming practice and biosecurity (such as 'all in and all out', clean and dry places', or feed back of diarrheal feces') can help prevent an infection (Nagy and Fekete, 1998).

To aid in alleviating the immediate post-weaning growth check, the early nursery diets will normally be high in nutrient density with high quality protein, palatability, and digestibility (van Beers-Schreurs and Bruininx, 2002).

1.3 Nursery diet composition

1.3.1 Typical early nursery diet composition

Following weaning, pigs are typically fed multiple-phase diets which decrease in diet complexity as the pigs get older (> 6.8 kg). Complex diets are diets that contain different specialty ingredients, such as whey, spray-dried animal plasma, FM, and other ingredients to maximize feed intake and nutritional value for young pigs. Specialty ingredients, such as spray-dried animal plasma, have been used to enhance performance

and increase feed intake and offer protection against *E. coli* with the plasma immunoglobulins (De Lange et al., 2010). However, there have been safety concerns with using spray-dried animal plasma (de Lange et al., 2010). The first phase typically contains SBM at approximately 12-15% of the diet, a lactose source at 20-25%, a high-quality fat source (i.e. choice white grease), soybean or corn oil, antibiotics, a zinc source (i.e. zinc oxide), and a high-quality protein source(s) that includes, but is not limited to, spray-dried animal plasma, FM, and blood cells. Lactose is a simple carbohydrate and is more easily broken down by the young pig compared to complex carbohydrates (van Beers-Schreurs and Bruininx, 2002). Whey is widely utilized in the early phases of the weaned pig diet as a highly digestible source of energy due to the greater lactase activity as opposed to other enzymes (i.e. amylase) at 3 wk of age (Kim and Allee, 2001) with dried whey typically consisted of 72% lactose, 8% ash, and 3% salt (Tokach et al., 1989). Pigs fed dried whey after weaning had greater ($P < 0.05$) gain in the first 5 wk (Tokach et al., 1989). Fermentable carbohydrates, such as fructooligosaccharides, resistant starch, and wheat bran, may be added to aid the weaned pig as a source of energy, influence composition and activity of GIT microbiota, and may provide some protection against post-weaning coli-bacillosis (Bikker et al., 2006). Fermentable dietary carbohydrates stimulate lactic acid producing bacteria, thus increasing lactic acid production, which may reduce coliform bacteria in the small intestine (Bikker et al., 2006). In the following phase, the high-quality protein source is typically reduced because pigs are already consuming feed and do not need more complex ingredients for feed intake stimulation. Soybean meal can now be increased during this phase and lactose can be decreased. The other components, such as zinc oxide and antibiotics are kept through the second phase to

promote health and growth performance. The interaction between diet complexity and feed intake immediately post-weaning should consider that: 1) feed intake will drive the growth performance in young pigs, 2) complex diets will improve the feed intake for the initial few wk post-weaning, and 3) as the impact of diet complexity and feed intake begins to reduce, diets should be formulated to be less complex to reduce the cost of feed per gain (DeRouchey et al., 2010).

1.3.2. Fishmeal, conventional soybean meal and further processed soybean meal

Fishmeal is a dried, powdered ingredient produced from pelagic fish that are captured for the primary purpose of producing FM or fish oil. Fishmeal can either be produced by using the entire fish and rendering it or by processing seafood by-products (Hardy and Tacon, 2002). Fishmeal is an excellent source of animal protein because it is easily digestible with an ideal composition of essential amino acids (AA) and can be comparable or greater in total concentration than SBM with lysine, methionine, tryptophan, threonine, and histidine (NRC, 2012). Fishmeal also has other essential nutrients, such as omega-3 fatty acids: eicosapentaenoic acid and docosahexaenoic acid (Hasan, 2012). In early nursery diets, FM is typically included as a high-quality protein source and to stimulate feed intake thus increasing gain (DeRouchey et al., 2010). Pigs fed diets including 8% menhaden FM had an 11.5% increase in ADG compared to pigs fed diets without FM (Stoner et al., 1990), but there are three main concerns with the use of FM in swine diets: 1) the relative expense 2) the limited supply, and 3) variability in measured growth responses from FM products (Jones et al., 2010). Fishmeal prices ranged from $1,500 to $2,400/ton, which was more than double the previous 15 yr (IndexMundi.com, August 1, 2014) and has been postulated to be due to the high demand

of FM from a limited and natural variation in supply (Hasan, 2012). The world's marine fisheries began a declining trend in 1996 and by 2011, 28.8% of the fish stocks were estimated to be at biologically unsustainable levels (FAO, 2014). An additional consideration with dietary FM inclusion is that the quality and benefits from FM depend on the type and species of fish, freshness of the fish prior to processing, the manufacturing process, and its effect on the total AA balance in the diet (Easter, 2001; Stoner el al., 1990). Fishmeal derived from mackerel and herring fish contain 9.8 and 5.4% greater total AA and 9.5 and 9.1%, respectively, greater lysine than FM derived from menhaden fish (Easter, 2001). The same author reported pigs had greater gain 1 wk post-weaning when fed FM derived from mackerel compared to menhaden FM and during wk 2 post-weaning, pigs fed diets containing mackerel or herring FM tended to have greater gain compared to the pigs fed diets containing menhaden FM. There was no additional benefit from FM, regardless of type, when fed to 3 or 4 wk post-weaning pigs (Easter, 2001). With increasing costs, sustainability concerns, and variability in FM, there is more interest to increase the utilization of plant-based protein sources.

Soybean meal is a coproduct from soybeans after cleaning, flaking, and oil extraction (NRC, 2012); it is the most widely used vegetable protein source for nonruminant animals because of its relatively high concentration of protein (44-49%) with greater levels of lysine, tryptophan, threonine, and histidine (NRC, 2012) providing a complementary essential AA profile with corn, and the dependable supply (Choct et al., 2010; Wang et al., 2011). After the oil extraction process, the nutritional composition of SBM is about 48% protein, 35-40% carbohydrates, 7-10% water, 5-6% minerals, and less than 1% fat on a dry matter basis (Choct et al., 2010). Soybean meal is a plant protein

that can compare to animal protein such as FM; thus, a swine diet can theoretically contain SBM as the sole source of protein following the early nursery phases (Shannon and Allee, 2010). Carbohydrates in SBM consist of approximately 10% free sugars, which can vary by location and by processing conditions, but the free sugars generally consist of 3-8% sucrose, 0.1-1.5% raffinose, and 1-6% stachyose (Choct et al., 2010). The SBM will increase in the diets as the pigs get older, but there may be negative digestive consequences if the immediate post-weaning diet consists of SBM at greater than 15% (DeRouchey et al., 2010). If a weanling pig's diet contains too much SBM the increased amount of non-starch polysaccharides will slow down the digesta rate of passage and can change the gut microflora to favor more fermentative bacteria. This may be a more favorable environment for ETEC to proliferate in the small intestine and become a factor in PWDS in the young pigs (Choct et al., 2010). Villous atrophy, crypt elongation, maldigestion and malabsorption, increased emptying rate through the stomach, and diarrhea are a few possible consequences with the introduction of soy protein to the weaned pig (Lallès, 1993). The introduction of SBM to a naïve pig can cause abnormalities in the digestive processes due to anti-nutritional factors (ANF; Li et al., 1991). Anti-nutritional factors can be defined as a substance generated from a natural feed stuff that hinders optimum utilization of that feed stuff by the inactivation of some nutrients or a diminution of the digestive process or metabolic utilization of the feed (Kumar, 1992). Some ANF in raw soybean, such as trypsin inhibitors and lectin, must be deactivated to optimize animal performance (Lallès, 1993). There are two major types of trypsin inhibitors known as the Kunitz and Bowman-Birk inhibitors, it is the Kunitz inhibitor that has been recorded to decrease protein digestion by inhibiting trypsin and

chymotrypsin in the pig (Herkelman et al., 1992). However, heating the soybeans helps to improve the nutritional value by destroying the trypsin inhibitors (Herkelman et al., 1992). Other ANF such as the soy protein antigens, glycinin and β-conglycinin, also pose a problem for young pigs due to the development of immune-mediated gut hypersensitivity (Lallès, 1993).

Sensitization to the antigens present in SBM causes an inflammatory response in the intestinal mucosa of the weaned pig (Li et al., 1991). Weaned pigs fed SBM had poorer performance, such as ADG and ADFI, compared to pigs fed skim milk-based diets in the first 14 d post-weaning (Li et al., 1991). They suggested the inclusion of SBM shortened intestinal villus height and hypertrophy in the crypt; thus, it decreased villus absorption area, disrupted digestive enzyme development, or disrupted the transport of nutrients at the surface area. The negative impact on the intestinal morphology can last for up to two wk after weaning (Lallès, 1993). Reports from in-vitro studies show β-conglycinin is more resistant than glycinin to porcine pepsin; however, most of the glycinin and β-conglycinin are denatured via thermal treatments (Lallès, 1993). Processing soy protein to combat its ANF has been in place from the early 1900s, which includes heat-treating, extruding, defatting, purifying, and relatively recently, fermenting (Min et al., 2004). Studies involving fermented soy protein feeding to weaned pigs began to readily appear in the early 2000s and it was believed the process increased nutrient digestibility and growth performance when fed to nursery pigs (Min et al., 2004). A common fermented soy protein product is produced using the fungus *Aspergillus Oryzae* under anaerobic conditions (Cho et al., 2007). That fungus fermented soy protein product was fed to weanling pigs by replacing 5, 10, or 15% of the conventional SBM in the basal

diet (Cho et al., 2007). The group reported similar performance between all the treatments (conventional and 3 inclusion rates of fermented soy protein) in the first 14 d, and by d 28 pigs fed the diet containing 15% inclusion of fermented soy protein treatment had greater feed efficiency (greater G/F) compared to the control, conventional SBM, treatment. Another fermented soy protein manufactured under anaerobic conditions using *Bacillus subtilis* was fed to weaned pigs and compared it to animal-based protein, including whey protein and FM (Yun et al., 2005). The same group reported pigs fed diets containing FM and whey protein concentrate had greater gain and feed efficiency (lower F/G) compared to pigs fed the fermented soy protein product in the first 14 d post-wean; however, in the following two wk, the growth performance variables previously listed were similar between these treatments. A different group fed a fermented SBM product, produced using *Aspergillus Oryzae* and *Bacillus subtilis* to weanling pigs in comparison to FM and dried porcine solubles (Jones et al., 2010). The group reported similar performance (ADG, ADFI, and G/F) between pigs fed diets containing FM and pigs fed diets containing the fermented SBM in the first 14 d post-wean. Additionally, the group reported the fermented SBM product could be fed with dried porcine solubles and improve growth performance compared to FM or diets with large concentrations of conventional SBM (Jones et al., 2010). A novel experimental further-processed SBM (MCSBM; Prairie AquaTech, Brookings, SD) has been introduced as a possible replacement for FM. The MCSBM is produced using the yeast-like fungal strain *Aureobasidium pullulans* in a unique incubation process (Sinn et al., 2016). Microbially-converted soybean meal has been shown to contain lower levels of ANF and higher digestibility of AA than conventional SBM when fed to fish (Sindelar, 2014). The cost of

MCSBM would be significantly lower than FM, approximately $600/ton for processing on top of the cost of SBM. Digestibility and performance trials in pigs utilizing MCSBM as a substitution for FM will be reported in the following chapters.

1.3.3 Antibiotics and dietary acidifiers

Antibiotic usage in animals have been used for over 50 yr and their main use is preventing the colonization of pathogenic bacteria in the intestine to promote overall health. Antibiotics are used therapeutically to treat a disease or subtherapeutically to promote growth performance (Richert, 2010). As of 2011, eleven antibiotics were approved for use in swine diets in the USA and when used as a growth promoter in weaned pigs diets, pig growth and feed efficiency are expected to improve by 16.4% and 6.9%, respectively (Cromwell, 2011). With respect to disease treatment or prevention, mortality is typically reduced from 4.3% to 2.0% when antimicrobials are added to the diets, but the mortality varies on disease pressure (Cromwell, 2011). Other factors that can affect antibiotic efficacy are management practices, including nutrition and health status. In feed antibiotics may not result in considerable improvement if the pigs are of high health status and managed optimally (Jacela et al., 2009). Although the mechanisms are not fully understood, there are several theories of possible mechanisms and it is likely that antibiotics help control the intestinal pathogens either directly or indirectly (Li et al., 2008). Antibiotics may inhibit infections caused by pathogenic bacteria, reduce bacterial metabolism that may negatively affect pig growth, and allow an increase of nutrient uptake through the intestinal wall by reducing bacterial growth in the gut (Jacela et al., 2009).

The main concerns with using antibiotics in feed for livestock are: 1) any antibiotic used must have the correct amount of withdrawal time before the animal is processed for human supply and 2) microbiota may develop a resistance to antibiotics making the antibiotic less effective in treatment of disease (Richert, 2010). An important point to keep in mind when considering removal of standard use of antibiotic inclusion in swine diets is that antibiotics do not provide additional nourishment for the pig; therefore, the absence of antibiotics from the diet is not expected to result in any nutritional deficiency (Jacela et al., 2009). Restricted use of feed grade antibiotics is not a new concept. For example, Sweden banned antibiotic growth promoters in 1986 (Stein, 2002). From the mid-2000s, the U.S. congress has considered restricting the subtherapeutic use of antibiotics in livestock feed and consumers were reported to willingly pay premium dollars for pork produced without antibiotics (Lusk et al., 2006). From January 1, 2017, a restriction was put in place for antibiotics in livestock feed by requiring a (Veterinary Feed Directive (VFD) except for the following antibiotics: Bacitracin, Bamvermycin, Carbadox, Tiamulin, and Narasin. The VFD was first introduced by the U.S. Food and Drug Administration in 2009 (MPB, 2016), and the VFD, in combination with the veterinary-client-producer-relationship concept, would help ensure medically important antimicrobial drugs would be used in feed according to label directions and only when it meets the animals' specific health needs (FDA, 2017). The final revision of the VFD in 2015 included the following changes that began in 2017: listed antibiotics must be ordered for the number of days in use, cannot be refilled unless labeled, and have a 6 mo maximum expiration date (MPB, 2016). Thus, alternatives to feed grade antibiotics have long been in consideration.

Following the discovery that weaned pigs have a limited capacity to secrete hydrochloric acid, acidifying weaned pig diets has been a topic of interest (Ravindran and Kornegay, 1993). Dietary acidifiers (organic, inorganic, or a mixture) have been used in swine diets to prevent mold growth in feed and provide antimicrobial and growth performance benefits. Some common dietary organic acids used are formic, acetic, propionic, butyric, citric, and lactic acid; these acidifiers can be used individually or combined with other acids. The addition of organic acids such as fumaric acid (Falkowski and Aherne, 1984; Giesting and Easter, 1985; Radecki et al., 1988; Giesting et al., 1991), and citric acid (Falkowski and Aherne, 1984; Henry et al., 1985) have improved pig performance. Usually between 1 and 2% of organic acids needs to be included to obtain a positive response (NRC, 2012). Propionic, fumaric, citric, and other organic acids have also been used to preserve feedstuffs (Falkowski and Aherne, 1984). The addition of fumaric or citric acid at 2%, pigs tended to have decreased feed intake but greater gain by 3% compared to pigs without dietary acidifier (Falkowski and Aherne, 1984). In that same study, dietary pH was measured and found to be reduced with 1 or 2% acidifier which reduced pH in the stomach and possibly increase pepsin activity. However, it may be too costly to add in organic acidifiers into the diet, and feed companies are limited to the inclusion rate for diet acidification (Kil et al., 2006). Inorganic acids, such as hydrochloric and phosphoric acids, have been studied for swine diets. There was a 13% and 12% increase in ADG and ADFI, respectively, with the addition of hydrochloric acid compared to the diets without acidification (Kil et al., 2006). On the contrary, in a previous study, the addition of hydrochloric acid resulted in feed intake and growth depression, which was theorized to be from the disruption of electrolyte balance by the

influx of chloride ions (Ravindran and Kornegay, 1993). There may be benefits to combining both organic and inorganic acids (Richert, 2010). Commercial acidifiers may contain a combination of both organic and inorganic acids and inclusion levels of the dietary acidifiers are generally low (NRC, 2012). The effects of the combination of these commercially available dietary acidifiers are difficult to predict because the amounts of the specific acids included are often proprietary information but positive responses to such products have been reported (Walsh et al., 2007). There was a numerical increase in ADG and decrease in F/G with pigs fed diets containing commercially available dietary acidifiers from d 0 to 7 post-weaning (Bergstrom et al., 1996). This particular trial utilized complex diets and high health status pigs weaned at 14 d; the authors suggested dietary acidifiers would provide benefits in growth performance if included in the first 7 d post-weaning or later in semi-complex diets if the pigs have not overcome stressors from weaning (Bergstrom et al., 1996). A few possible modes of actions for dietary acidifiers have been proposed, and the proposed modes mainly relate to maintaining a low gastric pH (Ravindran and Kornegay, 1993). Weaned pigs have difficulty in maintaining a low gastric pH, which may affect coliform proliferation and rate of emptying out of the stomach, ultimately resulting in diarrhea.

Weaned pigs must overcome many stressors in the immediate nursery phases, including separation from the dam, change in diet, new environment, and new pen mates. There are many advances in technology with feed additives including but not limited to: high-quality protein sources (animal or plant-based), feed grade antibiotics, and dietary acidifiers to aid in the pig's adaptation from weaning.

1.4 Hypothesis

From the immediately post-wean period through the early nursery, MCSBM may be used as a substitute for FM as a high-quality protein ingredient with less ANF compared to conventional SBM.

1.5 Research objectives

The objectives of this study were to 1) determine apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of MCSBM in comparison to Menhaden FM and 2) determine the effect of MCSBM as a replacement for FM in simple early nursery diets fed to newly weaned pigs on growth performance and gut health.

1.6 Purpose and significance of the study

Inclusion of high quality protein sources (i.e. FM) is important in weaned pig diets to manage digestive disturbances in the early post-weaning period, but there is concern over the long-term sustainability, increasing demands, and cost of FM supplies. MCSBM was engineered as a suitable high-quality plant protein substitution for FM. With the ANF concern in conventional SBM, MCSBM has been shown to have lower levels of ANF and greater digestibility of AA compared to conventional SBM. With the lower cost of MCSBM, this may reduce some of the cost in the early nursery complex diets, without compromising growth performance, and ultimately may reduce the cost of production.

Table 1.1 Levels of trypsin inhibitors, lectin, and antigenic proteins in soybean meal (adapted from Lallès, 1993)

	Soybean product			
Item	Raw	Defatted soybean meal	Soybean meal	Soy protein concentrate
Trypsin inhibitor, mg trypsin inhibited/g CP	40-120	15-60	6-8	2-6
Lectin	nd[1]	15	0-0.6	0-0.002
Antigenic proteins, mg/g CP				
Glycinin	nd	250-300	20-40	0-35
β-conglycinin	nd	150-200	15-35	0-25

[1]nd = not determined.

2.0 EVALUATION OF MICROBIALLY-CONVERTED SOYBEAN MEAL AS AN ALTERNATIVE TO FISHMEAL IN WEANED PIG DIETS

2.1 Abstract

An experimental MCSBM was evaluated as a replacement for FM. Assessment of feedstuffs should include estimation of digestibility as well as pig performance and in combination with dietary additives. Digestibility values determined in growing pigs may not apply to nursery pigs; thus, SID of AA in MCSBM and FM were determined using 30 ± 1.6 kg BW ileal-cannulated barrows (n = 6) and 9.8 ± 1.2 kg BW barrows (n = 37; serial slaughter). Experimental diets included MCSBM, FM, and nitrogen-free where FM and MCSBM were included as the sole protein source. The SID of AA was 3-5% lower in MCSBM than FM when fed to 30 kg pigs. The SID of Arg and Met was greater ($P < 0.05$) in MCSBM than FM when fed to 10 kg pigs. The SID of AA was 12-20% lower in FM when fed to 10 versus 30 kg pigs but only 3-9% lower in MCSBM. A total of 336 barrows and gilts were weaned at 21 d of age (initial BW 6.1 ± 0.8 kg) and used in a performance trial. Pens of pigs were assigned to one of 6 experimental diets (8 pens/diet in two blocks). Treatment diets were fed in Phase I (7 d) and Phase II (14 d) with all pigs fed a common Phase III diet (14 d). Experimental diets included: 1) negative control (NEG) containing corn, soybean meal and whey, 2) NEG + acidifier (NEGA), 3) NEG + FM (POS), 4) POS + acidifier (POSA), 5) NEG + MCSBM (MCSBM), and 6) MCSBM + acidifier (MCSBMA). The FM and MCSBM were included at 7.5% and 5.0% in Phase I and II diets, respectively. Diets were formulated to meet the standard nutrient requirements for weaned pigs. Pig BW and feed disappearance was measured weekly and fecal scores were measured daily for the first 14 d post-weaning as an indicator of

PWDS. Performance (BW, ADG, ADFI, and G/F) was not significantly different among treatments. Treatment for PWDS occurred on different days in each block. Analysis of fecal score was completed separately by block. Pigs fed the NEG diets had higher ($P=0.02$) fecal scores than pigs fed the POS diets on d 2 and 3 (block 1) and higher ($P<0.05$) than pigs fed MCSBM or POS diets and diets with dietary acidifier on d 6 and 3 (block 2). The MCSBM holds promise as an alternative for FM in nursery pig diets.

Key Words: digestibility, grower pigs, performance, protein sources, weaned pigs

2.2 Introduction

The weaning period presents new stressors to young pigs that typically results in low feed intake and a decrease in BW for several days immediately following weaning (Hötzel et al. 2011). Along with a loss of BW, an altered gut environment is favorable for the colonization of ETEC strains resulting in PWDS (Tsiloyiannis et al., 2001). It is important for diets in the immediate post-weaning period to include high quality ingredients, such as FM (DeRouchey et al. 2010), which are believed to increase feed intake and growth performance (Berrocoso et al., 2012). Dietary acidifiers have also been shown to benefit pig gut health and performance and may lower the severity or incidence of PWDS (de Lange et al. 2010).

There is heightened concern about overfishing of wild capture fisheries, and as of 2011, 28.8% of the world's recognized fish stocks were overfished (FAO, 2014). This over-harvest has included species used for FM production and when coupled with increasing global demand for FM has led to an unsustainable situation (Olsen and Hasan, 2012). Soybean meal is the most commonly used protein source in pig diets, but its use is

limited in young pig diets due to ANF, which influences PWDS (Friesen et al., 1993). Thus, it is important to find alternative high-quality protein sources that are cost-effective and contain minimal ANF.

Fermented soybean products have been evaluated as alternative protein sources (Jones et al. 2010) and an experimental MCSBM (Prairie AquaTech, Brookings, SD) has shown lower levels of ANF and higher digestibility of AA than conventional SBM when fed to fish (Sindelar, 2014). Microbially- converted soybean meal may be an alternative for FM in weaned pig diets but the digestible nutrient content when fed to pigs is unknown. Apparent ileal digestibility or SID using cannulated growing pigs is recommended as the standard method for measuring the digestible AA content of feedstuffs fed to swine (Stein et al., 2007). However, digestibility values determined from growing pigs do not necessarily apply to nursery pigs (Viljoen et al., 2000); therefore, two digestibility trials were conducted using 30 and 10 kg pigs because the goal was to evaluate MCSBM as a potential replacement for FM in nursery pigs.

Further, assessment of alternative feedstuffs should also include evaluation of pig performance and in combination with common dietary feed additives. The objectives of this study were to determine the digestible AA content of MCSBM and evaluate the use of MCSBM in nursery pig diets containing dietary acidifiers.

2.3 Materials and methods

All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (SDSU; IACUC #13-044A, 13-052A) and experiments were completed at the large animal research unit at

SDSU in Brookings, South Dakota. Barrows used for the digestibility trials were the offspring of Landrace × Large White sows mated with Hampshire × German Large White boars obtained from the SDSU swine research facility. Pigs used in the performance trial were obtained from a commercial piggery (Claremont Hutterite Colony, Castlewood, South Dakota, USA) and were of the same genetics as the SDSU swine research facility.

The experimental MCSBM evaluated in this study was provided by Prairie AquaTech (Brookings, SD, USA). The MCSBM production process involved incubating a pasteurized slurry of soybean meal and water with the fungus *Aureobasidium pullulans* for 4-5 days to convert sugars and oligosaccharides into fungal cell mass, while simultaneously degrading ANF such as trypsin inhibitors. Following incubation, the solids are recovered by centrifugation and dried (Brown and Gibbons, 2014). A commercial production facility is in the design phase and MCSBM will be priced competitively with FM.

2.3.1 Experiment 1

Animals, diets, and experimental design. For the 30 kg digestibility trial, AID and SID of CP and AA in MCSBM and FM were determined using 6 barrows (initial BW 30.0±1.6 kg) that were fitted with a simple T-cannula at the distal ileum adapted from Wubben et al. (2001). Barrows were adapted to individual metabolism pens equipped with two nipple drinkers and individual feeders housed in an environmentally controlled room (23±1 ^{o}C) for 7 d prior to surgery.

Following a minimum of 10 d post-surgery recovery period, barrows were randomly allotted to one of 3 experimental diets: MCSBM, FM, or nitrogen-free (Table

2.1) in a duplicated 3 × 3 Latin square design consisting of three 7-d collection periods (5 d diet adaptation and 2 d of 8 h digesta collection from 0830 to 1630 h). The MCSBM and FM were included as the sole protein source in their respective diets, and the N-free diet was used to estimate basal endogenous losses of CP and AA (Stein et al. 2007). Nutrient contents of the MCSBM and FM are listed in Table 2.2. Vitamins and minerals were included in all diets to meet or exceed current requirements for growing pigs (NRC, 2012), and titanium dioxide (titanium [IV] oxide, 98+%, anatase powder, Fisher Scientific, Pittsburgh, PA) was included at 0.1% in all diets as the indigestible marker (Zhu et al., 2005).

Barrows were fed at 2.5 x their daily maintenance energy requirement (106 kcal/kg of BW0.75; NRC, 2012), based on individual BW measured at the beginning of each collection period split in two equal allotments fed at 0800 and 1600 h. Digesta was collected according to the method of Cervantes-Pahm and Stein (2010), where 10 mL of 10% formic acid was added to each collection bag to prevent bacterial degradation (Fan et al., 1994), and bags were changed every 30 to 60 min, or near full capacity.

In the 10 kg BW digestibility trial, AID and SID of CP and AA in MCSBM and FM were determined using 37 barrows (9.8±1.2 kg BW) and the serial slaughter method. A total of 6 pigs were removed (2 from MCSBM, 4 from FM) from the dataset due to insufficient digesta available for collection resulting in n=10, 8, and, 13 for the MCSBM, FM, and N-free diets, respectively. Barrows were weaned at 28 d of age and housed in individual metabolism pens equipped with individual feeders and nipple drinkers in a 14 d feeding trial. Pigs were housed in an environmentally controlled room (28±1 ^{o}C) and

where necessary, supplemental heat was provided using heat lamps placed over individual pens.

Barrows were randomly assigned to one of the 3 experimental diets used in the 30 kg digestibility trial. Pigs were acclimated to weaning, metabolism pens, and experimental diets for 7 d, where experimental diets were included at an increasing ratio to a commercial starter pig diet until pigs were completely transitioned to experimental diets by the afternoon of d 7. Pigs were allowed ad libitum access to feed but to ensure maximal intake, fresh feed was provided daily at 08:00, 12:00, and 16:00 h. Prior to each feeding, any remaining orts were removed, weighed, and recorded. Water was available at all times throughout both digestibility trials.

Barrows were weighed on d 1 and on the morning of slaughter. On d 13 all feed was removed at 16:00 h and on d 14, beginning at 06:00 h in 15 min intervals (i.e., 06:00, 06:15, 06:30 h, etc.), pigs were given ad libitum access to their respective diets and were euthanized 4 h later (i.e. 10:00, 10:15, 10:30 h, etc.) by captive bolt and exsanguination. The entire gastrointestinal tract was excised, and ileal digesta was meticulously excised from a 30-cm section immediately proximal to the ileo-cecal junction and stored at -20 ^{o}C, pending analysis. In both of the digestibility trials, a daily subsample of the experimental diets was taken and pooled at the end of the trial for nutrient analysis.

2.3.2 Sample analyses and calculations

In the 30 kg digestibility trial, digesta was thawed, mixed with a Hobart mixer (Model A-200T, Troy, OH), and subsampled within animal, diet, and period, then lyophilized. In the 10 kg digestibility trial, the entire digesta sample for each pig was

lyophilized. All lyophilized digesta was finely ground prior to further analysis. Subsamples of test ingredients, experimental diets, and digesta were analyzed for AA by an institutional analytical lab (Agriculture Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO; Method 982.30 E(a,b); AOAC, 2006). Test ingredients and experimental diets were additionally analyzed for ash content, crude fat, crude fiber, and moisture by the Agriculture Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO; Method 942.05, 920.39 (A), 978.10, and 934.01, respectively; AOAC, 2006). Test ingredients, experimental diets, and digesta were analyzed for nitrogen (Rapid N III, Elementar, Hanau, Germany) and crude protein was calculated as nitrogen% x 6.25. Dry matter was measured using a THELCO laboratory oven (130DM, Chennai, India). Experimental diets and digesta were analyzed for titanium according to Short et al. (1996) with modifications. Briefly, samples were gently digested on a block heater (H2025-5A, Baxter Scientific Products, Soddy-Daisy, TN) for 20 h at 120 ºC and were vortex mixed every 30 min for 3 h, every 60 min for an additional 3 h, and again following the completion of digestion. Sample absorbance was measured with a spectrophotometer (SPECTRAMAX190, Molecular Devices, Sunnyvale, CA) at 408 nm.

2.3.3 Experiment 2

Animals, diets, and experimental design. A total of 336 pigs (184 barrows and 152 gilts) in two equal blocks were weaned at 21±1 d of age (initial BW = 6.1±0.8 kg) into 48 pens (n=7 pigs/pen). Each pen (1.8 by 1.2 m) contained one nipple drinker and 3-hole self-feeder to provide ad-libitum access to feed and water. Pens of pigs were randomly assigned to one of 6 experimental diets in a 35 d growth trial. The 6

experimental diets included: 1) a negative control (NEG; corn, SBM, and whey); 2) NEG plus acidifier (NEGA; KEMIN, Des Moines, Iowa, USA); 3) NEG plus FM (POS); 4) POS plus acidifier (POSA); 5) NEG plus MCSBM (MCSBM), and 6) MCSBM plus acidifier (MCSBMA; Table 2.3). Dietary acidifier was included at 0.2% in Phase I and II. Pharmacological levels (2,000 to 3,000 ppm) of zinc oxide are commonly added to nursery diets to reduce the occurrence of pathogens and is an effective means to minimize diarrhea (Lampromsuk et al., 2012). Because the impact of experimental diets on the incidence of diarrhea was evaluated, antibiotics and zinc oxide were not included in the diets. Diets were formulated to meet or exceed NRC (2012) requirements for weaned pigs on a SID AA and available phosphorus basis. The SID AA content in MCSBM and FM determined in the 30 kg digestibility trial was used to formulate the respective experimental diets. Metabolizable energy:ileal digestible Lys was maintained similarly across all diets for all phases. A 3-phase feeding program was used where experimental diets were fed from d 0 to 21 in 2 phases (Phase I, 7 d; Phase II, 14 d) and a common nursery diet was fed from d 22 to 35 (Phase III) post-weaning. Subsamples of all diets were collected routinely throughout the trial and stored at -20 °C, pending analysis.

Pigs were housed in an environmentally controlled room where the initial room temperature was 28±1 °C and reduced by 1 °C each week until d 21, and behavioral indices of cold stress (i.e., huddling) were monitored daily. Gender and BW were balanced across treatments and within pens as best as possible where the CV of the pen initial BW was ≤ 20%. A total of 32, 33, 33, 29, 29, and 28 barrows and 24, 23, 23, 27, 27, and 28 gilts were assigned to NEG, NEGA, POS, POSA, MCSBM, and MCSBMA, respectively. To determine the impact of treatment on gut health and microbiota, one pig

per pen nearest the mean BW was removed on d 7 and 21 for collection of ileal and cecal digesta and intestinal tissue. Within each collection day and treatment, equal number of barrows and gilts were used. Details on digesta and tissue collection will be reported in the next chapter.

Growth performance and diarrhea assessment. Pigs were weighed individually each week and pen feed disappearance was measured simultaneously. Due to errors associated with measuring feed disappearance, one pen fed the POS diet was removed from the data set prior to analysis. Incidence of diarrhea was recorded daily for each pen from d 1 to d 14 by three trained technicians according to the procedures and scoring system of Shu et al. (2001) based on a 6-point scale for fecal consistency: (1) hard and formed pellets; (2) non-formed pellets; (3) soft feces; (4) very soft, containing a small amount of water-like feces; (5) semisolid, containing more than 50% water-like feces, and (6) water-like feces. A mean daily score for each pen was calculated, and if a mean pen score was 5 or 6, or at least one observer gave a pen a score of a 6, it was considered PWDS requiring treatment. Diarrhea treatment followed SDSU Swine Research facility protocol; briefly, all pigs in pens identified as requiring treatment for PWDS were treated orally with Spectinomycin (Animal Medical Care, Brookings, SD) 2x/d for 3 d (2 mL/dose).

2.3.4 Data analysis

Digestibility data were analyzed using the PROC TTEST procedure of SAS (v9.3, SAS Inst. Inc., Cary, NC), where each pig was an experimental unit and ingredients were the fixed effects. Digestibility values from 30 and 10 kg pigs were compared using the PROC MIXED procedure of SAS (v9.3, SAS Inst. Inc.) where pig was the experimental

unit and ingredient, age, and their interactions were fixed effects. Performance data and daily pen fecal scores were analyzed using the PROC MIXED procedure of SAS (v9.3, SAS Inst. Inc.) with pen as the experimental unit in a randomized block design. Fixed effects were diet, dietary acidifier, and their interaction. For the performance data, pen nested within block and treatment was the random variable, and initial BW was used as a covariate. Treatment for PWDS was initiated on d 10 and 6 for blocks 1 and 2, respectively; therefore, for the fecal score assessment blocks were analyzed separately. The frequency of a pen receiving a PWDS treatment on d 1, or treated at all, was analyzed using the CATMOD procedure of SAS (v9.3, SAS Inst. Inc.). Statistical significance was established at $P<0.05$. For all ANOVA analyses, the Tukey-Kramer adjustment was used to test means separation where main effects were significant. All data was tested a priori for normality and homogeneity of variances using SAS (v9.3, SAS Inst. Inc.) and data is presented as least squares means±s.e.m.

2.4 Results and discussion

2.4.1 Experiment 1

The SID of isoleucine, leucine, lysine, methionine, and threonine were lower ($P<0.01$) in MCSBM than FM fed to 30 kg pigs (Table 2.4). The SID of CP, arginine, methionine, alanine, and glycine was greater ($P<0.05$) in MCSBM than FM fed to 10 kg pigs, and there were no differences in the SID of the other AA tested (Table 2.4). There was an age by ingredient interaction ($P<0.05$) where the SID of CP (88.8 vs 68.2 ± 5.67%), arginine (93.1 vs 72.2 ± 4.22%), lysine (93.8 vs 82.0 ± 2.16%), methionine (93.8 vs 76.7 ± 3.60%), phenylalanine (92.5 vs 81.2 ± 3.02%), alanine (92.0 vs 70.0 ± 4.28%), glutamate (93.4 vs 76.8 ± 4.28%), glycine (87.6 vs 46.5 ± 11.0%), proline (97.2 vs -32.01

± 45.0%), and tyrosine (92.4 vs 75.4 ± 4.76%) in FM was greater (P<0.05) when fed to 30 kg pigs than to 10 kg pigs; alternatively, the SID of CP and respective AA were equivalent in MCSBM when fed to 30 and 10 kg pigs (Table 2.4). While we did detect a lower SID of 5 essential AA in MCSBM the difference was between 3 and 5% lower and thus of limited significance on a practical diet formulation basis. However, it should be noted that due to differences in total AA content, the SID content of lysine and methionine were 33 and 50% lower in MCSBM than FM. As a result, use of crystalline lysine and methionine may be required when MCSBM is used in practical diet formulation.

The SID content (g/kg) of CP and all tested AA are listed in Table 2.4. The SID content (g/kg) of phenylalanine and cysteine were greater (P<0.05) in MCSBM than FM fed to 30 kg pigs. The SID content (g/kg) of lysine, methionine, threonine, alanine, and glycine were greater (P<0.05) in FM than MCSBM fed to 30 kg pigs. The SID content (g/kg) of arginine, phenylalanine, aspartate, cysteine, and glutamate were greater (P<0.05) in MCSBM than FM fed to 10 kg pigs. The SID content (g/kg) of lysine, methionine, threonine, and alanine were greater (P<0.05) in FM than MCSBM fed to 10 kg pigs. There was an age by ingredient interaction where the SID content of cysteine (7.02 vs 5.13 ± 0.57 g/kg) and serine (24.2 vs 20.9 ± 1.08 g/kg) were greater (P<0.05) in MCSBM when fed to 30 kg pigs than to 10 kg pigs; alternatively, the SID content (g/kg) of those respective AA in FM were equivalent when fed to 30 and 10 kg pigs. Additionally, SID content (g/kg) of CP, arginine (36.4 vs 28.2 ± 1.26 g/kg), lysine (48.1 vs 42.1 ± 0.94 g/kg), methionine (16.8 vs 13.7 ± 0.56 g/kg), alanine (36.9 vs 28.1 ± 1.44 g/kg), glutamate (80.8 vs 66.5 ± 3.81 g/kg), glycine (39.9 vs 21.2 ± 3.88 g/kg), proline

(29.5 vs -9.73 ± 13.7 g/kg), and tyrosine (18.4 vs 15.0 ± 0.97 g/kg) were greater ($P<0.05$) in FM when fed to 30 kg pigs than to 10 kg pigs; alternatively, SID content (g/kg) of those respective AA in MCSBM were equivalent when fed to 30 and 10 kg pigs. Age appeared to have a greater influence on SID and SID content for FM than MCSBM. The SID and SID content of AA in FM ranged from 12 to 20% lower when fed to 10 kg pigs but only 3 to 9% lower in MCSBM when fed to 10 kg pigs. While it is unclear why digestibility of FM was more influenced by age than MCSBM the digestibility of CP and AA in FM fed to 30 kg pigs is above or within the range of previously reported digestibility values for FM (Jørgensen et al. 1984; NRC 2012). Similarly, as the closest previously reported comparison for MCSBM, the SID of CP and most AA in MCSBM fed to 30 kg pigs were above the SID range reported for fermented soybean meal (Cervantes-Pahm and Stein 2010; NRC 2012).

Different methods of digesta collection were used for the 30 and 10 kg pigs; thus the effect of age must be compared with attention. Viljoen et al. (2000) reported higher digestibility values in cannulated pigs in comparison to the serial slaughter method using pigs of the same BW. At death, mucosal cells are shed into the intestinal lumen and influence the digestibility values of nitrogenous compounds (Badaway et al. 1957; Fell, 1961). Physically manipulating the intestine may also result in further sloughing of mucosal cells, contributing to the endogenous fraction of digesta (Viljoen et al. 2000). Great care was taken at the time of digesta collections in the 10 kg trial to limit physical manipulation of the intestine and minimize the impact of extraneous endogenous AA losses. Therefore, the differences between the two methods were expected to be minimal and the comparison between ages acceptable. Proline digestibility, and the associated

standard error estimate, may be used as an indirect evaluation of errors associated with digesta extraction, particularly for the protein-free diet. Proline makes up a large component of the AA content of endogenous losses and thus proline digestibility is heavily influenced by variables that increase endogenous loss estimates (i.e. low dietary protein, dietary fiber; Dilger et al. 2004). In the current study, the SEM of SID and SID content was 4-20 times greater than the other AA regardless of ingredient. The negative proline digestibility in the 10 kg pigs further indicates a high proportion of proline, compared to other AA, in the digesta from pigs fed the protein-free diet.

2.4.2 Experiment 2

There was no effect of ingredient, dietary acidifier, or their interaction on pig performance in Phase I, II, or III (Table 2.5). There was no block × dietary treatment effect on pig performance. However, pigs in block 1 were heavier ($P = 0.001$), had a greater daily feed disappearance ($P < 0.001$), daily gain ($P = 0.001$), and G/F ($P = 0.002$) in Phase I than pigs in block 2. During Phase II, block 1 pigs had a greater G/F ($P < 0.02$). Pigs in block 2 had a greater daily gain ($P = 0.005$) and G/F ($P = 0.001$) during Phase III than pigs in block 1.

In block 1, pigs fed the NEG diets had greater ($P = 0.02$) fecal scores than pigs fed the POS diets on d 2 and on d 3 (Fig 2.1). In block 2, pigs fed diets without dietary acidifier had higher ($P = 0.047$) fecal scores than pigs fed diets with dietary acidifier on d 3 (Fig 2.2). In block 2, pigs fed the NEG diets had higher ($P = 0.01$) fecal scores than pigs fed either MCSBM or POS diets on d 6. There was no interaction between ingredient and dietary acidifier on fecal score. A total of 13, 25, 25, 25, 13, and 25% of pens fed NEG, NEGA, POS, POSA, MCSBM, and MCSBMA, respectively, were treated on d 1

of PWDS intervention, and 63, 63, 50, 75, 38, and 63% of pens fed NEG, NEGA, POS, POSA, MCSBM, and MCSBMA, respectively, were treated at least once for PWDS.

In the present study, dietary inclusion of FM or MCSBM reduced the severity, and inclusion of MCSBM reduced the incidence, of PWDS in weaned pigs but neither FM nor MCSBM influenced growth performance compared to pigs fed NEG. We observed reduced fecal scores in pigs fed diets containing MCSBM or FM compared to the control pigs, which is in agreement with Song et al. (2010) who noted that replacement of conventional soybean meal with fermented soybean meal reduced diarrhea in weaned pigs. The reduced severity of diarrhea may be due to degradation of β-conglycinin and glycinin in MCSBM as a result of further processing. Glycinin and β-conglycinin have been shown to stimulate hypersensitivity in the young pig (Li et al. 1990). It was expected that pigs fed diets containing MCSBM and FM would have improved performance compared to pigs fed the NEG diets due to the reduced ANF. Anti-nutritional factors, such as trypsin inhibitors, raffinose, and stachyose in conventional SBM have been shown to reduce nursery pig performance and nutrient digestibility at high inclusion rates in the diet; thus, processed soy proteins were developed to reduce the levels of ANF (Min et al., 2004). On average, levels of ANF in conventional soybean meal range from 2,596 to 6,090 TIU/g trypsin inhibitor, 0.98% raffinose, and 3.07% stachyose (Choct et al., 2010). The analyzed content of MCSBM included 68.88 TIU/g trypsin inhibitor, 0.00% raffinose, and 0.02% stachyose. The levels of trypsin inhibitor, raffinose, and stachyose supplied by each experimental diet was calculated based on analyzed values for MCSBM and a mean value for the basal SBM used in producing the MCSBM (Prairie AquaTech, Brookings, SD) and corn (NRC,

2012). Trypsin inhibitor, raffinose, and stachyose levels were found to be 66.8, 50.0, and 50.0%, respectively, lower in the MCSBM and POS diets compared to the NEG diets. All experimental diets were formulated to contain similar metabolizable energy and metabolizable energy:SID lysine ratio; therefore, the similar performance can be explained by similar diet formulation, which may overcome the ANF. Other fermented soy protein (i.e. HP 300 and PepSoyGen; Hamlet Protein A/S, Horsens, Denmark and Nutraferma Inc., North Sioux City, South Dakota, respectively) have been evaluated as alternative protein ingredients for nursery pig diets with varying results (Jones et al. 2010; Min et al. 2004). Min et al. (2004) reported an increase in pig performance (overall daily gain and weekly feed intake) when diets included fermented soy protein. The improved response was explained by greater digestibility in complete diets containing processed soy protein. In our study, digestibility of MCSBM and FM were determined a priori and used to formulate performance study diets containing equivalent SID AA. Jones et al. (2010) reported no performance advantage when fed fermented soy protein was included in the diet alone or in combination with FM but improved performance (daily gain and G/F) when included in diets containing plasma products. Experimental diets reported by Jones et al. (2010) are similar in ingredient content as those used in the current study, particularly the control (equivalent to the NEG diet) which was formulated with an expectation of reduced pig performance. In the current study, weekly and overall growth performance in pigs fed the NEG diet was above that reported by Jones et al. (2010) and Min et al. (2004) for all treatments indicating excellent growth performance of all pigs in our study. As discussed previously, the lack of response observed with MCSBM in the current study may be related to an equivalent supply of digestible

nutrients among all treatments, as well as, the high growth performance observed in all pigs. The lower fecal scores observed in pigs fed diets with dietary acidifier may also be due to the lower pH in the upper intestinal tract. Inclusion of acidifier in the diet is suggested to inhibit proliferation of pathogenic microorganisms by lowering gastric pH (Tsiloyiannis et al. 2001). Tsiloyiannis et al. (2001) reported a benefit in PWDS in weaned pigs by feeding diets with organic acidifiers. The present study found the inclusion of dietary acidifier provided no additional benefit to growth performance but did have a positive effect on PWDS, as pigs fed diets with dietary acidifier had lower fecal scores than pigs fed diets without dietary acidifiers. According to Bergstöm et al. (1996), the inclusion of organic acids in starter diets may not provide additional growth benefits when the diets contain high levels of milk products. Experimental diets used in the present study contained high levels of whey which may have minimized the effects of the dietary organic acids.

2.5 Conclusion

The results of the current study support our hypothesis that MCSBM is a suitable alternative for FM in nursery pig diets because of the similar ileal digestible CP and AA content in MCSBM compared for FM and the positive impact on PWDS. As well, the lesser reduction in digestibility estimates for MCSBM when fed to the 10 kg pigs further supports our conclusion that MCSBM is a suitable alternative for FM. While there was no interaction between MCSBM and dietary acidifier, possible interactions with other key ingredients should be explored. The maximum level of MCSBM to achieve optimal gain/cost of feed should also be assessed.

Table 2.1 Composition and analyzed nutrient content of Experiment 1 diets, as-fed basis.

Item	MCSBM [1]	Fishmeal[1]	Nitrogen-free
Ingredient (%)			
Cornstarch	56.1	62.2	62.8
Solka floc	-	-	5.00
Sucrose	10.0	10.0	20.0
MCSBM	25.0	-	-
Soya oil	2.00	2.00	4.00
Fishmeal	-	20.0	-
Magnesium sulfate	-	-	0.20
Potassium carbonate	-	-	0.60
Limestone	1.00	0.50	0.80
Dicalcium phosphate	0.80	0.50	1.00
Sodium chloride	0.50	0.50	0.50
Monocalcium phosphate	1.50	1.20	2.00
Vitamin premix[2]	1.50	1.50	1.50
Mineral premix[3]	1.50	1.50	1.50
Titanium dioxide	0.10	0.10	0.10
Analyzed composition (%)			
Dry matter	93.8	93.2	93.6
CP	14.7	13.2	0.85
Ash	5.79	7.92	5.43
Crude fat	1.64	3.41	1.60
Crude fiber	1.21	0.41	1.35
Analyzed amino acid content (%)			
Arginine	3.95	3.91	-
Histidine	1.53	1.53	-
Isoleucine	2.85	2.62	-
Leucine	4.74	4.56	-
Lysine	3.68	5.13	-
Methionine	0.83	1.79	-
Phenylalanine	2.95	2.35	-
Threonine	2.36	2.67	-
Valine	2.95	3.00	-
Alanine	2.63	4.01	-
Aspartate	6.57	5.80	-
Cysteine	0.85	0.52	-
Glutamate	9.89	8.66	-
Glycine	2.57	4.56	-
Proline	3.04	3.04	-
Serine	2.68	2.47	-
Tyrosine	2.06	1.99	-
Total	56.1	58.61	-

[1]Microbially-converted soybean meal (MCSBM) and Menhaden fishmeal provided by Prairie AquaTech (Brookings, South Dakota, USA).

[2]Provided per kg of the complete diet: 329,736 IU vitamin A supplement, 49,467 IU vitamin D_3 supplement, 1,650 IU vitamin E supplement, 1.32 mg vitamin B_{12} supplement, 132 mg menadione as menadione dimethylpyrimidinol bisulfite, 297 mg riboflavin supplement, 1,815 mg D-pantothenic acid as D-calcium pantothenate, 1,650 mg niacin supplement, 33 mg folic acid, 99 mg pyridoxine as pyridoxine hydrochloride, 99 mg thiamine as thiamine mononitrate, and 5.12 mg biotin.

[3]Provided per kg of the complete diet: 1.65 g Zn as zinc sulfate, 1.65 g Fe as ferrous sulfate, 440 mg Mn as manganese sulfate, 160 mg Cu as basic copper chloride, 3.6 mg I as ethylenediamine dihydroiodide, and 3 mg of Se as sodium selenite.

Table 2.2 Analyzed nutrient content of the microbially-converted soybean meal and Menhaden fishmeal used in Experiments 1 and 2, as-fed basis

	Ingredient[1]	
Item	MCSBM	Fishmeal
Gross energy (kcal/kg)	4,688	4,577
Dry matter (%)	95.8	92.9
CP (%)	58.4	63.2
Ash (%)	7.6	20.9
Crude fat (%)	0.00	9.22
Crude fiber (%)	5.74	0.39
Indispensable amino acids (%)		
Arginine	3.95	3.91
Histidine	1.53	1.53
Isoleucine	2.85	2.62
Leucine	4.74	4.56
Lysine	3.68	5.13
Methionine	0.83	1.79
Phenylalanine	2.95	2.35
Threonine	2.36	2.67
Valine	2.95	3.00
Dispensable amino acids (%)		
Alanine	2.63	4.01
Aspartate	6.57	5.80
Cysteine	0.85	0.52
Glutamate	9.89	8.66
Glycine	2.57	4.56
Proline	3.04	3.04
Serine	2.68	2.47
Tyrosine	2.06	1.99
Total amino acids	56.1	58.6

[1]Ingredients provided by Prairie AquaTech (Brookings, South Dakota, USA).

Table 2.3 Composition and nutrient content of Experiment 2 diets, as-fed basis

	Phase I[1]			Phase II[1]			Phase III[1]
Item	NEG	POS	MCSBM	NEG	POS	MCSBM	
Ingredient (%)							
Corn	33.6	39.8	38.3	44.8	50.6	49.6	60.8
Soybean meal	37.3	25.0	25.0	40.7	30.7	30.7	34.2
Fishmeal	-	7.49	-	-	4.99	-	-
Whey powder	25.0	25.0	25.0	9.98	9.98	9.98	-
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calcium phosphate	1.22	0.35	1.25	1.55	0.95	1.55	1.60
Limestone	0.85	0.47	0.87	0.92	0.70	0.95	1.05
Sodium chloride	0.30	0.30	0.30	0.35	0.35	0.35	0.35
L-Lysine-HCL	0.11	0.05	0.21	0.14	0.15	0.25	0.30
DL-Methionine	0.14	0.11	0.07	0.13	0.12	0.10	0.14
L-Threonine	0.04	0.04	0.03	0.06	0.08	0.07	0.13
L-Tryptophan	-	0.04	0.02	-	0.03	0.02	-
L-Valine	-	-	0.04	-	-	0.04	-
Trace mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.25
MCSBM	-	-	7.48	-	-	4.99	-
Titanium dioxide	0.20	0.20	0.20	0.20	0.20	0.20	-
Analyzed composition (%)							
Dry matter	90.9	91.4	90.8	89.9	89.9	89.8	89.9
CP	24.3	23.9	23.7	23.9	23.0	22.5	22.7
Ash	6.71	6.70	6.80	6.56	6.07	6.32	5.76
Crude fat	2.44	2.95	1.41	1.97	2.64	1.79	2.43
Crude fiber	1.71	1.65	1.83	2.61	2.59	2.70	3.21
Lysine	1.60	1.48	1.53	1.51	1.57	1.50	1.44
Formulated content							

Metabolizable energy (MJ/kg)	13.9	14.2	14.3	13.8	14.0	14.1	13.8
Lysine:metabolizable energy (g/MJ)	0.97	0.95	0.94	0.98	0.96	0.96	0.90

[1]Experimental diets were fed from d 0 to 7 (Phase I) and from d 8 to 21 (Phase II) post-weaning. All pigs received a common Phase III diet d 22 to 35. A mixture of acids were used for dietary acidifier (KEMIN, Des Moines, Iowa, USA), which was included at 0.2% in NEG, POS, and MCSBM diets to create NEGA, POSA, and MCSBMA diets in Phase I and II. The microbially-converted soybean meal (MCSBM) and Menhaden fishmeal used in Phase I and II were provided by Prairie AquaTech (Brookings, South Dakota, USA).

[2]Provided per kg of the complete diet: 165 mg Zn as zinc sulfate, 165 mg Fe as ferrous sulfate, 43.5 mg Mn as manganese sulfate, 16.5 mg Cu as basic copper chloride, 0.36 mg I as ethylenediamine dihydroiodide, and 0.3 mg of Se as sodium selenite.

[3]Provided per kg of the complete diet: 11,002 IU vitamin A supplement, 1,651 IU vitamin D_3 supplement, 55.1 IU vitamin E supplement, 0.044 mg vitamin B_{12} supplement, 4.4 mg menadione as menadione dimethylpyrimidinol bisulfite, 9.91 mg riboflavin supplement, 60.6 mg D-pantothenic acid as D-calcium pantothenate, 55.1 mg niacin supplement, 1.1 mg folic acid, 3.3 mg pyridoxine as pyridoxine hydrochloride, 3.3 mg thiamine as thiamine mononitrate, and 0.171 mg biotin.

Table 2.4 Standard ileal digestibility and standardized digestible crude protein and amino acid content in microbially-converted soybean meal and Menhaden fishmeal fed to 30 and 10 kg pigs (Experiment 1; as-fed basis)

	30 kg				10 kg			
Item	MCSBM	Fishmeal	SEM	*P*-Value	MCSBM	Fishmeal	SEM	*P*-Value
Standardized ileal digestibility (%)								
CP	90.3	88.8	1.88	0.653	81.0	68.2	4.20	0.050
Arginine	94.5	93.1	1.86	0.640	83.9	72.2	3.03	0.017
Histidine	89.6	92.0	0.893	0.119	80.7	75.7	2.49	0.169
Isoleucine	91.0	94.8	0.704	0.004	83.1	84.2	2.05	0.690
Leucine	91.6	95.1	0.676	0.005	83.3	85.1	1.95	0.530
Lysine	88.8	93.8	0.618	<0.001	85.6	82.0	1.65	0.150
Methionine	91.0	93.8	0.580	0.010	87.0	76.7	2.85	0.020
Phenylalanine	90.9	92.5	0.660	0.125	83.1	81.2	2.39	0.575
Threonine	88.1	92.7	0.889	0.007	77.8	79.3	2.70	0.713
Valine	90.4	92.6	0.772	0.072	82.5	81.6	2.14	0.756
Alanine	90.5	92.0	1.43	0.520	81.8	70.0	3.26	0.020
Aspartate	84.7	86.6	0.797	0.128	73.5	74.0	2.67	0.883
Cysteine	82.5	83.2	2.96	0.888	60.3	66.4	6.54	0.518
Glutamate	91.7	93.4	0.733	0.167	84.7	76.8	3.30	0.113
Glycine	91.5	87.6	3.45	0.531	81.3	46.5	8.26	0.009

Proline	104.0	97.2	20.2	0.838	-3.4	-32.0	32.0	0.541
Serine	90.3	89.2	1.39	0.587	77.9	76.8	3.29	0.810
Tyrosine	94.9	92.4	2.39	0.489	82.8	75.4	3.43	0.148
Standardized digestible content (g/kg)[2]								
CP	527	561	25.7	0.782	473	431	21.1	0.513
Arginine	37.3	36.4	1.25	0.955	33.1	28.2	1.03	0.012
Histidine	13.7	14.1	0.370	0.904	12.4	11.6	0.304	0.306
Isoleucine	25.9	24.8	0.536	0.476	23.7	22.1	0.440	0.070
Leucine	43.4	43.4	0.874	1.00	39.5	38.8	0.717	0.898
Lysine	32.7	48.1	0.708	<0.001	31.5	42.1	0.581	<0.001
Methionine	7.55	16.8	0.421	<0.001	7.22	13.7	0.346	<0.001
Phenylalanine	26.8	21.7	0.595	<0.001	24.5	19.1	0.488	<0.001
Threonine	20.8	24.7	0.673	0.002	18.4	21.2	0.552	0.007
Valine	26.7	27.8	0.617	0.576	24.3	24.5	0.506	0.998
Alanine	23.8	36.9	1.09	<0.001	21.5	28.1	0.896	<0.001
Aspartate	55.6	50.2	1.63	0.111	48.3	42.9	1.33	0.042
Cysteine	7.02	4.33	0.448	0.001	5.13	3.45	0.368	0.017
Glutamate	90.7	80.8	2.88	0.098	83.8	66.5	2.36	<0.001
Glycine	23.5	39.9	2.93	0.003	20.9	21.2	2.40	1.00

Proline	31.6	29.5	10.3	0.999	-1.02	-9.73	8.48	0.886
Serine	24.2	22.0	0.852	0.295	20.9	19.0	0.699	0.238
Tyrosine	19.5	18.4	0.730	0.683	17.1	15.0	0.599	0.099

[1]Digesta was collected using t-cannulas in the distal ileum with 30 kg pigs (n = 6/diet) and by the serial slaughter method with 10 kg pigs (n = 10 and 8 for MCSBM and FM, respectively). The MCSBM and fishmeal were provided by Prairie AquaTech (Brookings, South Dakota, USA).

[2]The SID of CP and each AA was multiplied by the concentration of CP and corresponding AA for each ingredient to calculate the standardized ileal digestible CP of AA of each ingredient (as-fed basis).

Table 2.5 Effect of dietary inclusion of microbially-converted soybean meal and Menhaden fishmeal with or without dietary acidifier on growth performance of weaned pigs in Experiment 2.

	NEG		MCSBM		POS			*P*-Value[2]		
Item	- Acid	+ Acid	- Acid	+ Acid	- Acid	+ Acid	SEM	Ingredient	Acid	Block
BW (kg)										
d 0	6.09	6.17	5.94	6.14	6.34	6.08	0.302	0.865	0.975	0.154
d 7	6.43	6.34	6.37	6.45	6.49	6.42	0.072	0.711	0.688	0.001
d 21	9.88	9.70	9.54	9.90	9.78	9.95	0.163	0.747	0.483	0.394
d 35	17.8	18.1	17.6	18.1	18.1	18.1	0.313	0.792	0.290	0.851
d 0 to 35	12.3	12.4	12.1	12.5	12.4	12.5	0.226	0.746	0.368	0.484
Daily feed disappearance (g)										
Phase I	114	112	114	114	111	114	7.03	0.985	0.978	<0.001
Phase II	442	434	429	449	437	450	14.0	0.943	0.532	1.00
Phase III	818	891	850	856	906	894	23.1	0.174	0.325	0.515
d 0 to 35	527	552	535	545	559	561	15.6	0.338	0.342	0.379
Daily gain (g)										
Phase I	44.6	30.3	35.4	47.8	52.6	42.2	10.4	0.711	0.688	0.001
Phase II	346	330	323	348	331	348	12.9	0.965	0.485	0.067
Phase III	607	639	618	634	631	621	13.1	0.978	0.325	0.005
d 0 to 35	390	394	383	401	395	396	10.7	0.926	0.393	0.839
Gain:feed										
Phase I	0.381	0.232	0.307	0.357	0.449	0.334	0.083	0.656	0.385	0.002
Phase II	0.817	0.802	0.777	0.829	0.797	0.826	0.017	0.912	0.189	0.018
Phase III	0.745	0.722	0.731	0.760	0.708	0.697	0.018	0.134	0.925	0.001
d 0 to 35	0.702	0.662	0.663	0.720	0.689	0.678	0.023	0.918	0.928	0.059

[1]Experimental diets were fed in Phase I (d 0 to 7 post-weaning) and Phase II (d 8 to 21) in a 3-phase feeding study. All pigs received a common Phase III diet from d 22 to 35. The negative control diet (NEG) was based on corn, soybean meal, and whey with decreasing inclusion of why from Phase I to II. Microbially-converted soybean meal and fishmeal were added to the NEG diet at 7.5% at the expense of conventional soybean meal in Phase I and 5% in Phase II to create the MCSBM and POS diets, respectively. The MCSBM

and fishmeal were provided by Prairie AquaTech (Brookings, South Dakota, USA). Dietary acidifier was included in experimental diets at 0.2%.

[2] Ingredient main effect refers to NEG, MCSBM, or FM. There was no block by main effects interaction and no ingredient by acid interaction.

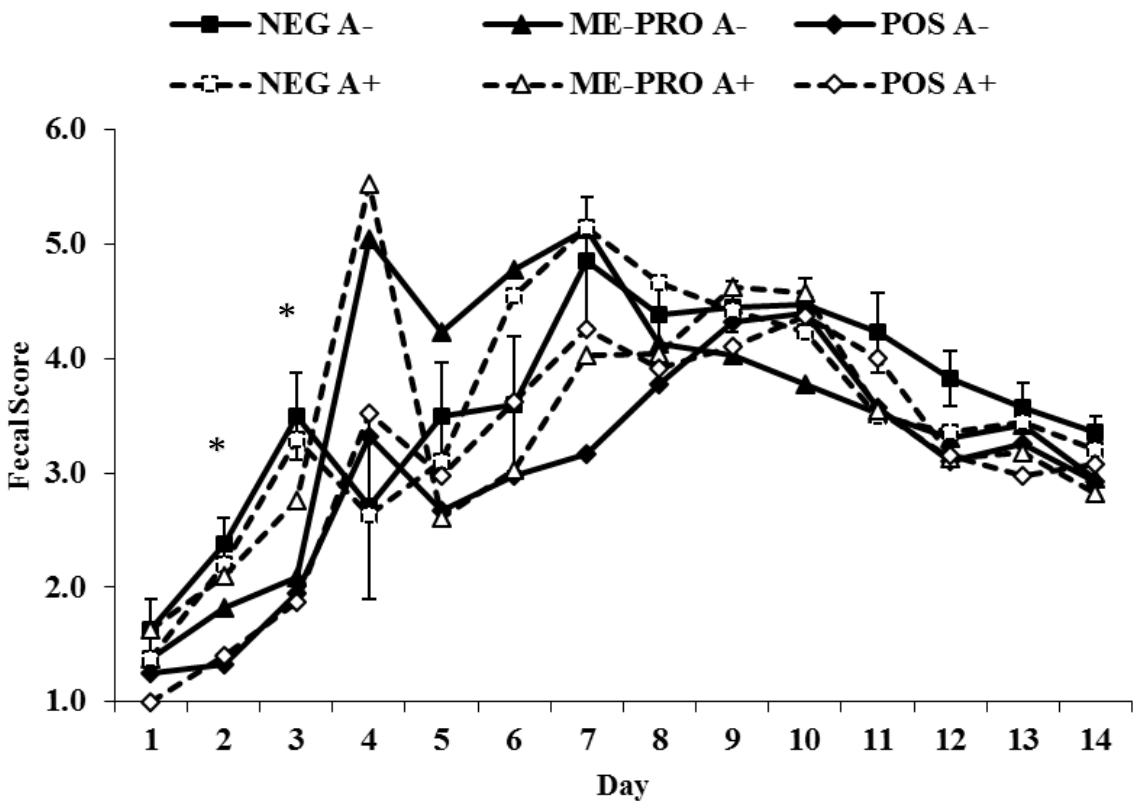

Figure 2.1 Daily pen fecal scores of weaned pigs in block 1 fed diets containing microbially-converted soybean meal (MCSBM) or Menhaden fishmeal (POS) compared with a negative control diet (NEG) with or without dietary acidifier (MCSBM A+, POS A+, NEG A+)[1].

[1]Treatment for diarrhea began on day 10.

[*]Significant difference ($P < 0.05$).

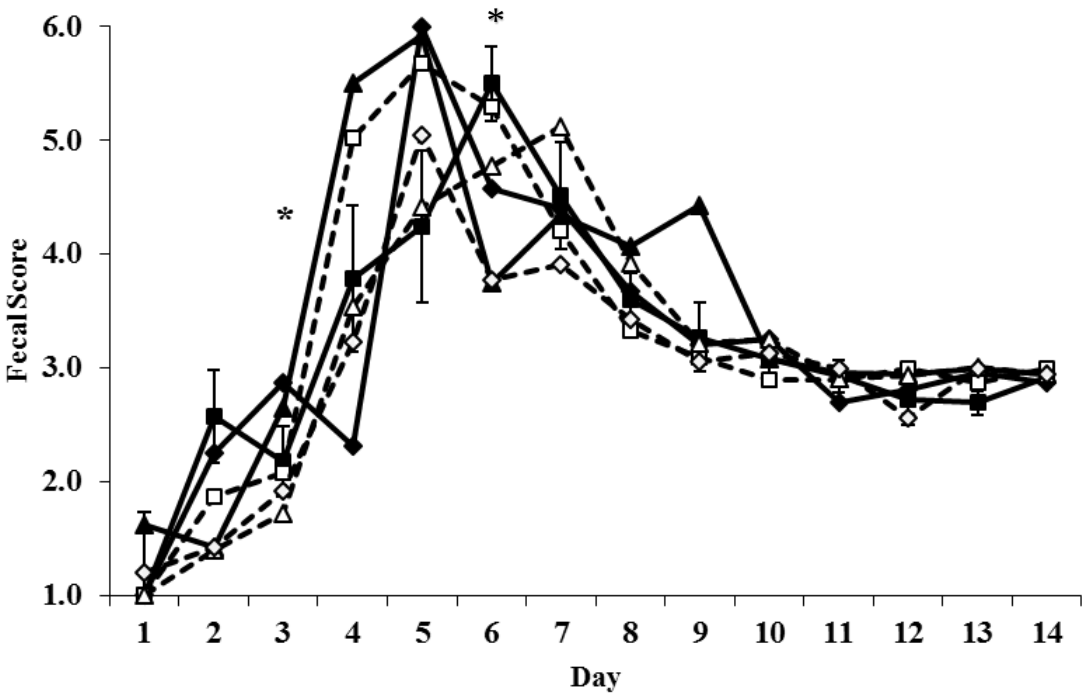

Figure 2.2 Daily pen fecal scores of weaned pigs in block 2 fed diets containing microbially-converted soybean meal (MCSBM) or Menhaden fishmeal (POS) compared with a negative control diet (NEG) with or without dietary acidifier (MCSBM A+, POS A+, NEG A+)[1].

[1]Treatment for diarrhea began on day 6.

[*]Significant difference ($P < 0.05$).

3.0 EFFECTS OF MICROBIALLY-CONVERTED SOYBEAN MEAL AND FISHMEAL IN WEANED PIG DIETS ON GASTROINTESTINAL FUNCTION

3.1 Abstract

An experimental MCSBM was evaluated in comparison to FM and conventional SBM on gut function. Gut morphology and function assessment including measuring pH along the GIT, VH, CD, VH:CD, goblet cell area: total cell area, Ki-67, and inflammation and mucin scoring in the stomach and upper duodenum. This study was conducted simultaneously with the growth performance study in Chapter 2. Digesta and tissue samples were collected at the end of Phase I and II. The pH was measured for both Phase I and II, but only Phase I samples were measured for the other listed measurements. At the end of Phase I and II, there was an effect on location ($P < 0.0001$), where the lowest pH was observed in the stomach and steadily increased until the ileum followed by a slight dip in the cecum. Pigs fed NEG and POSA diets had similar ($P > 0.10$) pH from the stomach to duodenum at the end of Phase I. At the end of Phase II, pigs fed NEGA diets had similar ($P > 0.10$) pH from the stomach to the duodenum. A total of 6, 7, 3, 7, 5, and 4 ileal digesta samples tested positive for *E. coli* for NEG, NEGA, MCSBM, MCSBMA, POS, and POSA, respectively, at the end of Phase I out of 8 observations from each treatment (7 observations for POSA). One sample each from MCSBMA and POS treatments were positive for *E. coli* K88. There was no difference between treatments for all gut health and function measurements, which includes VH, CD, VH:CD, goblet cell area, cell proliferation, and inflammation and mucin scoring.

The MCSBM holds promise as an alternative for FM in nursery pig diets.

Key Words: fishmeal, gut morphology and function, MCSBM, and weaned pigs

3.2 Introduction

Conventional weaning is one of the most stressful events for a pig. Low feed intake and a subsequent decrease in BW is typical for several days immediately following weaning (Hötzel et al., 2011). As well, young pigs may be subjected to degenerative changes in the anatomy and physiology of the small intestine such as villous atrophy (Pluske et al., 1997; Tsiloyiannis et al., 2001). Enterotoxigenic *E. coli* strains can take advantage of this altered gut environment resulting in PWDS (Tsiloyiannis et al., 2001).

It is important for diets in the immediate post-weaning period to include high quality ingredients, such as FM (DeRouchey et al., 2010), which are believed to increase feed intake (Berrocoso et al., 2012). However, over-harvest of species included in FM, coupled with increasing global demand, has led to an unsustainable situation (Hasan, 2012). Soybean meal is the most commonly used protein source in pig diets, but its use is limited in young pig diets due to ANF, which influences PWDS (Friesen et al., 1993). There are also a multitude of studies with blends of dietary acidifiers that have directly or indirectly benefitted pig gut health via anti-microbial activity, lowering the pH of the upper small intestine, or by enhancing the mucosal integrity and function (De Lange et al., 2010). Fermented SBM products have previously been evaluated as alternative protein sources (i.e. Jones et al., 2010). Inclusion of MCSBM in nursery pig diets resulted in lower dietary ANF content and lower pig fecal scores compared to pigs fed diets containing conventional SBM (evaluated in Chapter 2); therefore, we hypothesized that pigs fed MCSBM and FM in the early post-weaning diets would have similar gut function but better gut function than pigs fed conventional SBM.

3.3 Materials and methods

Experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at SDSU (IACUC #13-052A) and was conducted at the large animal research unit at SDSU in Brookings, South Dakota. Pigs used in the trial were the offspring of Landrace × Large White sows mated with Hampshire × German Large White boars and were obtained from a commercial piggery (Claremont Hutterite Colony, Castlewood, South Dakota, USA).

3.3.1 Animals, diets, and experimental design

This study was conducted simultaneously with the growth performance trial in Chapter two. Details of the animals, diets, and experimental design can be found in Section 2.3.3.

3.3.2 Sample collection, preparation, and analyses

On d 6 and 7 (end of Phase I) and d 20 and 21 (end of Phase II) in each block, one mean pig per pen, based on average BW and performance (balancing for gender between treatments), was euthanized for sample collection. Pen fecal grab samples were collected and stored at -20 ^{o}C on d 5 to 7 and 19 to 21 for future microbiota analysis; microbiota analysis was not completed within this thesis project. Beginning at 0900 h on collection days, the selected pig in each pen was stunned by captive bolt gun and euthanized by exsanguination; a total of 12 pigs/d were euthanized in 20 min intervals to ensure collection of fresh tissue. The abdominal cavity was opened, and the entire gastrointestinal tract was carefully excised from the carcass. Digesta pH was measured at six locations along the GIT: stomach, proximal duodenum, mid jejunum, distal ileum,

cecum, and colon using a Thermo Orion digital pH meter (Model #360; Hogentogler&Co, Inc, Columbia, MD). The stomach was clamped off at both the cardia and pyloric regions before removal, digesta content from the cardiac region was emptied into a beaker for pH measurement. A tissue sample from the pyloric and corpus regions was dissected, gently rinsed with saline and blotted dry, placed in buffered formalin for a minimum of 24 h, and sent to the ADRDL (Brookings, South Dakota) to be dehydrated, infiltrated with paraffin wax, and stored for future preparation. The duodenum (30 cm from the pylorus), jejunum, and ileum (last 30 cm from the ileo-cecal junction) were separated by clamps, and the small intestine was detached from the large intestine. Upper duodenum was separated into two sections: one section was gently rinsed with saline, blotted dry, and snap frozen in liquid nitrogen for further analysis, and the other section was gently rinsed with saline, blotted dry, placed in buffered formalin for a minimum of 24 h, and sent to the South Dakota State University pathology lab (ADRDL) to be dehydrated, infiltrated with paraffin wax, and stored for future preparation. Digesta and fecal samples were gently extracted from a 30 cm section proximal to the ileo-cecal junction and mid-cecum and rectum, respectively, and snap frozen in liquid nitrogen for further *E. coli* and microbiome assessment.

The ADRDL prepared unstained and stained slides from the stomach and duodenum tissue samples using the H&E. The VH, CD, and VH:CD were measured and calculated using the H&E stained duodenum slides by utilizing 10 sections per tissue and taking an average of the values to give a mean slide value. Additional unstained slides were sent to an outside lab to be stained with a PAS (Department of Animal Science, University of Minnesota, St. Paul, MN). Standard immunohistochemical techniques were

used to stain for Ki-67 by an outside lab (Department of Animal Science, University of Minnesota, St. Paul, MN). Stomach and duodenum slides were blindly assessed for mucin type with the PAS by assigning a score to 3 sections per tissue and taking the average of the scores to give a mean slide score, where mucin color was scored on a 5-point scale with 1 - pink (neutral) and 5 - blue (acidic). For goblet cell assessment, duodenum tissue samples were sectioned and mucin staining was performed using the Alcian blue/PAS kit (Newcomer Supply, Middleton, WI) following the manufacturer's instructions. The area occupied by goblet cells was calculated by dividing the area (μm2) of mucin stained (blue or dark purple) by the total tissue area per field captured at 200X magnification. Images were captured using a BX53F Olympus microscope and images were analyzed using Olympus Cellsense software (Olympus America Inc., Center Valley, Pennsylvania). The percent of the tissue occupied by goblet cells was assessed per field in five fields (chosen randomly) in the slide and averaged per animal for group comparisons. Stomach and duodenum tissue samples were prepared by an outside lab (Department of Animal Science, University of Minnesota, St. Paul, MN) for inflammatory analysis. Briefly, 4 μm tissue sections were mounted on charged slides, deparaffinized, and rehydrated by sequential inmmersion in xylene, 100% ethanol, 80% ethanol and phosphate buffer saline. Antigen retrieval was performed by boiling the slides in 10 mM sodium citrate buffer pH 6.0. The DAB Substrate Kit (Abcam, Cambridge, MA) was used for immunohistochemistry staining following the manufacturer instructions with an additional 1 h blocking step with goat serum 1:200 followed by primary antibody Anti-CD45 (1:500 Abcam, Cambridge, MA) incubation for 2 h at room temperature. Clusters of inflammatory cells in all fields occupied by tissue

were identified at 200X magnification and counted. Scores were assigned to each field as: 1 - clusters only at the base of the gland, 2- clusters displacing tissue up to the neck of the gland and 3 - inflammatory infiltrate fully displacing the gland. Each field was evaluated by multiplying the number of clusters by the score per field. For intestinal samples, the same procedure was followed with an additional score of 4 given to fields where the inflammatory infiltrate was invading the submucosa. Field values were average per animal for final group comparisons.

3.3.3 Data analyses

The pH, histology, and immunohistochemistry data were analyzed using the PROC MIXED procedure of SAS (v9.3, SAS Inst. Inc.) with pen as the experimental units. Fixed effects were diet, dietary acidifier, and their interaction; GIT location and its interaction with the other main effects was included as fixed effects for the pH data. Pen nested within block and treatment was the random variable. The frequency of *E. coli* presence in the ileum was analyzed using the CATMOD procedure of SAS (v9.3, SAS Inst. Inc.). Statistical significance was established at $P < 0.05$. For all ANOVA analyses, the Tukey-Kramer adjustment was used to test means separation where main effects were significant. All data was tested a priori for normality and homogeneity of variances using SAS (v9.3, SAS Inst. Inc.) and data is presented as least squares means ± SE.

3.4 Results and discussion

At the end of Phase I, the mean pH for the GIT sections were 3.98, 5.38, 6.47, 7.08, 6.17, and 6.41 in the pyloric region of the stomach, duodenum, jejunum, ileum, cecum, and colon, respectively (Figure 3.1). There was an effect of location on pH ($P <$

0.0001), where the lowest pH was observed in the pyloric region of the stomach, and each measured location in the small intestine significantly increased ($P < 0.05$) until the ileum then decreased in the cecum. Within GIT location, there was no effect of ingredient, dietary acidifier, or their interaction on GIT pH (Table 3.1). Within treatments, pigs fed NEG and POSA diets had similar pH ($P > 0.10$) between the stomach and duodenum while pigs fed NEGA, MCSBM, MCSBMA, and POS diets had lower ($P < 0.05$) pH in the stomach compared to the duodenum. Pigs fed MCSBM, MCSBMA, and POS diets had similar ($P > 0.10$) pH between the duodenum and jejunum, while pigs fed NEG, NEGA, and POSA diets had lower ($P < 0.05$) pH in the duodenum compared to the jejunum. At the end of Phase II, the mean pH for the GIT sections were 4.18, 5.55, 6.25, 6.74, 5.60, and 5.93 in the pyloric region of the stomach, duodenum, jejunum, ileum, cecum, and colon, respectively. There was an effect of location on pH ($P < 0.0001$), where the lowest pH was observed in the pyloric region of the stomach, and each measured location in the small intestine significantly increased ($P < 0.05$) until the ileum then decreased in the cecum. However, the pH in the cecum was similar ($P > 0.10$) to the pH in the duodenum. This pH data follows a similar pattern to the data reported by Risley el. Al (2014). Within GIT location, there was no effect of ingredient, dietary acidifier, or their interaction on GIT pH. Within treatments, pigs fed NEGA diets had similar ($P > 0.10$) pH values between the stomach to the duodenum while pigs fed NEG, MCSBM, MCSBMA, POS, and POSA diets had lower ($P < 0.05$) pH in the stomach compared to the duodenum. Pigs fed NEG, NEGA, MCSBMA, POS, and POSA diets had similar ($P > 0.10$) pH in the duodenum compared to the jejunum, while pigs fed MCSBM diets had lower ($P < 0.05$) pH in the duodenum compared to the jejunum. The

presence or absence of *E.coli* in ileal digesta collected at the end of Phase I were analyzed using the CATMOD procedure of SAS and are reported in Table 3.1. Insufficient digesta was collected from 1 pig fed POSA, but all other means are based on 8 observations. A total of 6, 7, 3, 7, 5, and 4 digesta samples were positive for *E. coli* in pigs fed NEG, NEGA, MCSBM, MCSBMA, POS, and POSA, respectively. Of the digesta samples positive for *E. coli*, 2 samples (1 each from MCSBMA and POS) were positive for the K88 strain. For all diets, ≥50% of samples from all diets tested positive for STa, except for NEGA, which only 28.6% test positive for STa. For all diets, ≥50% of samples from all diets tested positive for STb. At the end of Phase I, the mean pH values for pyloric and cecum were within the ranges reported by Risley et al. (2014). The group fed simple diets without milk products and with or without dietary organic acidifiers and suggested feeding diets containing dried whey lowers the pH of the diet compared to diets without the addition of dried whey (Risley et al., 2014). At the end of Phase II, the mean pH value for the pyloric region was 13.4% greater than the range reported by Risley et al. (2014). Milk products can reduce pH in the gastrointestinal contents (Burnell et al., 1988) due to the lactose converting into lactic acid (Ravindran et al., 1993). Makkink et al. (1994) reported no change in gastric pH with pigs fed skimmed milk powder, soy protein concentrate, SBM, or FM, and concluded protein source did not influence gastric pH at 3 or 6 d post-wean. In that same study, Mikkink et al. (1994) reported soybean-based diets resulted in lower gastric pH 10 d post-wean compared to diets without soybean. However, all diets consisted of SBM and dried whey, which may explain why there was no effect of dietary treatment, with or without diet acidification, on gastric pH at d 7 or 21 post-wean.

There was no effect of ingredient, dietary acidifier, or their interaction on VH, CD, VH:CD, goblet cell area, Ki-67, and inflammation or mucin scoring in the stomach or duodenum at the end of Phase I (Table 3.2). The objective for this current study was to evaluate the effects of the MCSBM compared to FM on gut function and morphology in the immediate post-weaning period and if these two products would result in better gut function compared to conventional SBM. Samples were first assessed at the end of Phase I for these gut health measurements because the weaned pig will often not meet energy requirements for maintenance in the first 24 to 48 h post-wean due to limited feed intake but will meet energy requirements 8 to 14 d post-weaning (Lallés et al., 2004). Due to the lack of differences observed with morphology and immunohistochemistry at the end of Phase I, Phase II samples were not assessed at that time. Villous atrophy, crypt elongation, maldigestion and malabsorption, increased emptying rate through the stomach, and diarrhea are more than likely possible consequences with the introduction of soy protein to the weaned pig (Lallès, 1993). In the current study, diets with the greater concentration of SBM (NEG and NEGA) did not have as detrimental effects as those listed previously, which may be explained by the addition of corn. The consequences for the introduction to soy protein may be minimalized by feeding a corn-based diet (Dunsford et al., 1988). In addition, VH in pigs across all dietary treatments in this study were within the range for VH in pigs at 7 d post-wean reported by Li et al. (1990) who also fed diets containing similar levels of milk products. Goblet cells (also known as mucous cells in the stomach) reside in the epithelium and secrete a viscoelastic coat presumed to contribute to the mucosal protection (Kindon et al., 1995). Among the goblet cells are the mucin glycoproteins that contribute to the gel formation in conjunction with

mucus-producing cells (Kindon et al., 1995). The lack of difference in the goblet cell area is consistent with the lack of difference between treatments in the mucin scores within the stomach and upper duodenum. Ki-67 is a marker of intestinal cell proliferation where the positive (brown) cells can be counted and calculated with total cell count as an indicator of tissue growth (Wiyaporn et al., 2013). The lack of difference between treatments for cell proliferation is consistent with the lack of difference in the morphological assessment of the duodenum (i.e. VH, CD, and VH:CD) for all treatments. Inflammation in the GIT may be influenced by multiple factors, including pathogenic microorganisms or toxins, antigenic activity (i.e. feedstuffs), or levels of alkaline phosphatase (Campbell et al., 2013). Alkaline phosphatase is an enzyme that aids in detoxifying pathogenic bacterial lipopolysaccharide endotoxins that cause inflammation in the GIT (Campbell et al., 2013). Inflammation occurs when the intestinal barrier is disrupted by said factors, which increases permeability for the harmful toxins, bacteria, or antigens to cross over the barrier, and thus, resulting in malabsorption, diarrhea, and reduced growth performance (Campbell et al., 2013). The lack of differences in inflammation in the stomach and upper duodenum observed in the current study, suggests minimal influence of the experimental diets on inflammation in the upper GIT.

3.5 Conclusion

From the results of the current study, we may accept part of our original hypothesis that pigs fed diets containing MCSBM would have similar gut function in comparison to pigs fed diets containing FM but cannot conclude that either ingredient resulted in better gut function compared to conventional SBM.

Table 3.1 Digesta pH at six locations along the gastrointestinal tract and presence of *Escherichia coli* in ileal digesta of weaned pigs fed diets containing microbially-converted soybean meal (MCSBM) and Menhaden fishmeal (FM) with or without dietary acidifier.

	NEG diets[1]		MCSBM diets[1]		POS diets[1]		
Item	- Acid	+ Acid	- Acid	+ Acid	- Acid	+ Acid	SEM[4]
Location, d 7 pH[2]							
Stomach	4.20[a]	3.99[a]	3.92[a]	3.97[a]	3.74[a]	4.11[a]	0.243
Duodenum	5.09[a]	5.56[b]	5.60[b]	5.62[b]	5.65[b]	4.74[a]	0.243
Jejunum	6.36[b]	6.62[c]	6.23[b]	6.28[b]	6.57[b]	6.78[b]	0.262
Ileum	6.95	6.88	7.29	7.10	7.10	7.15	0.262
Cecum	5.95	6.29	6.21	6.00	6.27	6.31	0.243
Colon	6.24	6.59	6.49	6.28	6.51	6.37	0.243
Location, d 21 pH[2]							
Stomach	4.56[a]	4.16[a]	3.86[a]	4.00[a]	4.17[a]	4.31[a]	0.189
Duodenum	5.61[b]	5.17[a,b]	5.37[b]	5.55[b]	5.53[b]	6.08[b]	0.252
Jejunum	6.11[b]	6.17[b]	6.36[c]	6.43[b]	6.17[b]	6.25[b]	0.189
Ileum	6.52	6.54	6.54	7.07	6.79	6.98	0.203
Cecum	5.65	5.62	5.63	5.51	5.76	5.45	0.189
Colon	5.96	6.06	6.01	5.78	5.94	5.84	0.189
Presence of *E. coli*, %[3]							
Yes	75.0	87.5	37.5	87.5	71.4	50.0	0.530
No	25.0	12.5	62.5	12.5	28.6	50.0	0.530

[1]Experimental diets were fed in Phase I (d 0 to 7 post-weaning) and Phase II (d 8 to 21) in a 3-phase feeding study. The negative control diet (NEG) was based on corn, soybean meal, and whey with decreasing inclusion of why from Phase I to II. The MCSBM and FM were added to the NEG diet at 7.5% at the expense of conventional soybean meal in Phase I and 5% in Phase II to create the MCSBM and POS diets, respectively. The MCSBM and FM were provided by Prairie AquaTech (Brookings, South Dakota, USA). Dietary acidifier was included in experimental diets at 0.2%.

[2]At the end of Phase I and Phase II, d 7 and 21, respectively, one pig representing the average pen weight was selected and humanely euthanized to measure pH at six locations along the GIT and collection of digesta samples from the ileum. The pH data were analyzed using the PROC MIXED procedure of SAS (v9.3, SAS Inst. Inc.) with pen as the experimental units. Fixed effects were diet, dietary acidifier, and their interaction; GIT location and its interaction with the other main effects was included as fixed effects for the pH data.

[a,b,c] Means within a column (stomach, duodenum, and jejunum only) without a common superscript letter differ ($P < 0.05$).

[3]Frequency of *E. coli* (yes or no) was analyzed using the PROC CATMOD procedure of SAS with diet, acid, and its interaction in the model on d 7 samples.

[4]Reported SEM is largest among measurements.

Table 3.2 Histology and immunohistochemistry results from 7 d post-weaned pigs fed microbially-converted soybean meal (MCSBM) and Menhaden fishmeal (FM) with or without dietary acidifier for one week.

	NEG diets[1]		MCSBM diets[1]		POS diets[1]			*P*-value[4]	
Item[2]	- Acid	+ Acid	- Acid	+ Acid	- Acid	+ Acid	SEM[3]	Diet	Acid
Villus height, μm	300	281	305	263	286	271	37.3	0.920	0.326
Crypt depth, μm	282	259	311	276	263	258	33.1	0.519	0.360
VH:CD, μm:μm	1.09	1.10	1.10	1.09	1.19	1.07	0.127	0.952	0.684
Goblet cell area:total cell area, μm^2:μm^2	0.032	0.041	0.038	0.035	0.030	0.035	0.005	0.663	0.368
Ki-67, %									
Positive:total cells	61.2	61.6	60.9	59.4	63.3	63.1	6.07	0.801	0.901
Inflammation scores									
Stomach	1.48	1.41	1.30	1.33	0.62	1.34	0.381	0.292	0.389
Duodenum	0.896	1.06	0.880	0.566	0.750	0.772	0.271	0.605	0.844
Mucin scores									
Stomach bottom	3.45	3.45	3.17	2.99	2.93	3.31	0.564	0.687	0.861
Stomach neck	3.49	3.78	3.67	3.85	3.24	3.76	0.613	0.881	0.438
Stomach pit	3.04	2.87	2.67	3.38	2.99	3.67	0.415	0.555	0.194
Brunner's gland	2.63	2.98	2.64	2.82	2.29	2.99	0.450	0.909	0.218

[1]Experimental diets were fed in Phase I (d 0 to 7 post-weaning). The negative control diet (NEG) was based on corn, soybean meal, and whey. The MCSBM and FM were added to the NEG diet at 7.5% at the expense of conventional soybean meal in Phase I to create the MCSBM and POS diets. The MCSBM and FM were provided by Prairie AquaTech (Brookings, South Dakota, USA). Dietary acidifier was included in experimental diets at 0.2%.

[2]At the end of Phase I, d 7, one pig representing average pen weight was selected and humanely euthanized for GIT tissue samples to measure GIT function at various locations. The histology and immunohistochemistry data were analyzed using the PROC MIXED procedure of SAS (v9.3, SAS Inst. Inc.) with pen as the experimental units. Fixed effects were diet, dietary acidifier, and their interaction.

[3]Reported SEM is largest among measurements.

[4]*P*-values not listed for diet × acid ($P > 0.10$).

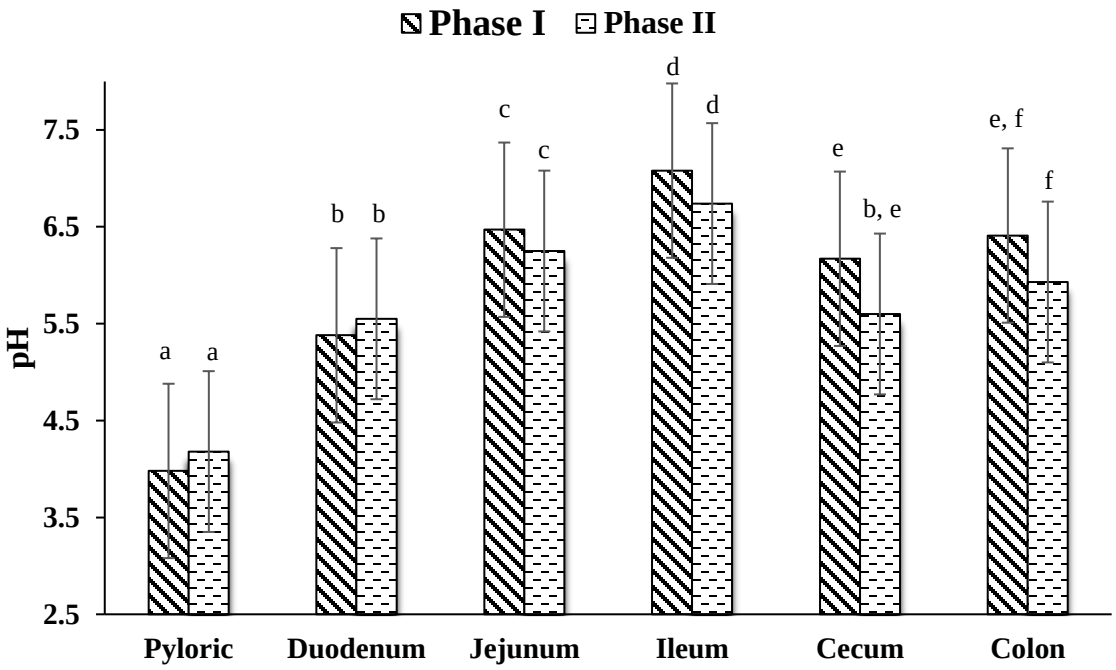

Figure 3.1 Average results of pH at six locations along the gastrointestinal tract of weaned pigs fed NEG, MCSBM, and Menhaden fishmeal (FM) diets with or without dietary acidifier. The negative control diet (NEG) was based on corn, soybean meal, and whey with decreasing inclusion of why from Phase I to II. Microbially- converted soybean meal and fishmeal were added to the NEG diet at 7.5% at the expense of conventional soybean meal in Phase I and 5% in Phase II to create the MCSBM and POS diets, respectively. The MCSBM and fishmeal were provided by Prairie AquaTech (Brookings, South Dakota, USA). Dietary acidifier was included in experimental diets at 0.2%. Pigs were humanely euthanized on d 7 and d 21 for Phase I and II, respectively.

[a,b,c]Means within phase without a common superscript letter differ ($P < 0.05$).

4.0 GENERAL DISCUSSION

The objective of this project was to evaluate MCSBM as a possible substitution for FM in early nursery diets immediately following weaning. The purpose for looking at a possible replacement for FM are due to several concerns: 1) the relative expense, 2) sustainability of fish populations, and 3) variability in measured growth responses from different FM products (Jones et al., 2010). We can't feed high concentrations of conventional SBM due to the ANFs and triggered hypersensitivy in the naïve pig; therefore the hypothesis was that MCSBM can replace FM as a high-quality protein source with similar, if not better, growth performance in the early nursery phases possibly at a lower cost, and pigs fed both MCSBM and FM would have better gut health and function compared to pigs fed only conventional SBM.

In the 30 kg digestibility study, FM had a greater ($P < 0.05$) SID of Lys, Thr, and Met compared to MCSBM. However, in the 10 kg digestibility study showed the SID of CP was greater ($P = 0.05$) for MCSBM compared to FM. The SID of Met was greater ($P = 0.02$) for MCSBM compared to FM for 10 kg pigs, and there were no differences in SID of Lys and Thr. From a practical formulation perspective based off 30 kg digestibility results, diets with MCSBM would need synthetic AA supplementation if replacing FM. However, from the 10 kg digestibility results, MCSBM would be a suitable replacement for FM without the need for supplementation of additional synthetic AA. For the growth performance trial in Chapter 2, there were no differences in performance between any of the treatments, which can be explained by maintaining metabolizable energy: ileal digestible Lys across treatments. Early nursery diets were formulated to be simple (without complex high-quality ingredients such as spray dried

plasma) with more conventional SBM than the recommended ≤ 15% (DeRouchey et al., 2010) to compare MCSBM and FM with less factors than the typical complex nursery diets used in commercial production. MCSBM and FM appeared to provide a benefit in relation to PWDS with lower fecal scores compared to pigs fed diets containing conventional SBM alone. The lower fecal scores for the MCSBM and FM diets may be explained by the lower concentration of conventional SBM in those diets and could suggest less use of feed grade antibiotics as opposed to diets with high concentrations of conventional SBM. If a weaned pig's diet contains too much conventional SBM, the digesta rate may be slowed and favorable for the proliferation of pathogenic *E. coli* in the upper small intestine (Choct et al., 2010). With similar gut morphology and function between treatments presented in Chapter 3, we cannot conclude MCSBM or FM is a better ingredient over conventional SBM, but it's important to note the MCSBM and FM diets did contain a fairly high concentration of conventional SBM. According to Sindelar (2014), MCSBM was shown to have lower levels of ANFs, which contribute to PWDS, and greater digestibility of AA compared to conventional SBM, but because all diets contained at least 25% conventional SBM in Phase I diets, we can't conclude pigs benefitted from MCSBM with lower levels of ANF. It may be beneficial to the weaned pig to get introduced to soy protein via MCSBM due to the lower levels of ANFs, but it may not be realistic from a cost perspective because MCSBM is at least $600/ton more than conventional SBM. However, because the pigs fed diets containing conventional SBM alone performed similarly to the pigs fed diets containing MCSBM or FM, we cannot conclude MCSBM or FM provided additional growth performance benefits when added to the early nursery diets. This conclusion is in contrast with data reported by

Stoner et al. (1990), where Menhaden FM inclusion resulted in improved ADG and ADFI, but that study also evaluated increasing levels of FM with decreasing levels of SBM. The addition of whey and utilizing a corn-based diet may explain why dietary acidifiers did not make an impact in this trial. The similar results from Chapter 3, evaluation of gut function, in combination with the similar growth performance in chapter 2 support our hypothesis that MCSBM is a suitable alternative for FM in nursery pig diets. This data also suggest pigs fed complex diets containing MCSBM may perform similarly compared to pigs fed complex diets with FM, which would need to be evaluated. However, to completely evaluate the gut function and morphology of MCSBM compared to FM, simple diets with similar buffering capacity, where the conventional SBM, MCSBM, and FM are the only protein sources should be fed with sample collection either at weaning or within 24 h post-wean, d 7, and d 14 d post-wean.

5.0 BIBLIOGRAPHY

Badaway, A. M., R. M. Campbell, D. P. Cuthbertson, and B. F. Fell. 1957. Changes in the intestinal mucosa of the sheep following death by humane killer. Nature. 180:756-757.

Bergstrom, J. R., J. L. Nelssen, M. D. Tokach, R. D. Goodband, J. A. Loughmiller, R. E. Musser, and J. Nessmith, W. B. 1996. An Evaluation of Several Diet Acidifiers Commonly Utilized in Pig Starter Diets to Improve Growth Performance Swine Day.

Berrocoso, J. D., M. P. Serrano, L. Cámara, P. G. Rebollar, and G. G. Mateos. 2012. Influence of diet complexity on productive performance and nutrient digestibility of weanling pigs. Anim. Feed Sci. Tech. 171:214-222.

Bikker, P., A. Dirkzwager, J. Fledderus, P. Trevisi, I. le Huerou-Luron, J. Lalles, and A. Awati. 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. J. Anim. Sci. 84:3337-3345.

Brooks, P., C. Moran, J. Beal, V. Demeckova, A. Campbell, M. Varley, and J. Wiseman. 2001. Liquid feeding for the young piglet. In: The weaner pig: nutrition and management. Proceedings of a British Society of Animal Science Occasional Meeting, University of Nottingham, UK, September 2000. p 153-178.

Brown, M., and W. Gibbons. 2014. High value protein replacers for aquaculture feed and industrial products from defatted soybean white flakes and meal. In: USB Domestic Opportunities Meeting, Little Rock, AR

Bruininx, E. M. A. M., G. P. Binnendijk, C. M. C. van der Peet-Schering, J. W. Schrama, L. A. den Hartog, H. Everts, and A. C. Beynen. 2002. Effect of creep feed consumption on individual feed intake characteristics and performance of group-housed weanling pigs. J. Anim. Sci. 80:1413-1418.

Cera, K., D. Mahan, R. Cross, G. Reinhart, and R. Whitmoyer. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. J. Anim. Sci. 66:574-584.

Cervantes-Pahm, S. K., and H. H. Stein. 2010. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88:2674-2683.

Cho, J. H., B. J. Min, Y. J. Chen, J. S. Yoo, Q. Wang, J. D. Kim, and I. H. Kim. 2007. Evaluation of FSP (Fermented Soy Protein) to Replace Soybean Meal in Weaned Pigs: Growth Performance, Blood Urea Nitrogen and Total Protein Concentrations in Serum and Nutrient Digestibility. Asian. Austral. J. Anim. 20:1874-1879.

Choct, M., Y. Dersjant-Li, J. McLeish, and M. Peisker. 2010. Soy oligosaccharides and soluble non-starch polysaccharides: A review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian. Austral. J. Anim. 23:1386-1398.

Cranwell, P. D. 1985. The development of acid and pepsin (EC 3. 4. 23. 1) secretory capacity in the pig; the effects of age and weaning.

Cromwell, G. L. 2011. Feed supplements: Antibiotics. In: D. E. Ullrey, C. K. Baer and W. G. Ponds (eds.) Encyclopedia of Animal Science. p 391-393. CRC Press, Boca Raton, FL.

Davis, M., S. Sears, J. Apple, C. Maxwell, and Z. Johnson. 2006. Effect of weaning age and commingling after the nursery phase of pigs in a wean-to-finish facility on growth, and humoral and behavioral indicators of well-being. J. Anim. Sci. 84:743-756.

De Lange, C. F. M., J. Pluske, J. Gong, and C. M. Nyachoti. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livest. Sci. 134:124-134.

Deplancke, B., and H. R. Gaskins. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. The American journal of clinical nutrition 73:1131S-1141S.

DeRouchey, J. M., R. D. Goodband, M. D. Tokach, J. L. Nelssen, and S. S. Dritz. 2010. Nursery swine nutrient recommendations and feeding management. In: D. J. Meisinger (ed.) National Swine Nutrition Guide. U.S. Pork Center of Excellence, Ames, IA. p 65-79.

Dilger, R., J. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724.

Dritz, S. S., K. Q. Owen, J. L. Nelssen, R. D. Goodband, and M. D. Tokach. 1996. Influence of Weaning Age and Nursery Diet Complexity on Growth Performance and Carcass Characteristics and Composition of High-Health Status Pigs from Weaning to 109 Kilograms. J. Anim. Sci. 74:2975-2984.

Easter, R. A. 2001. Nutritional value of fish meals in the diet for young pigs. J. Anim. Sci. 79:11p.

Efird, R. C., W. D. Armstrong, and D. L. Herman. 1982. The development of digestive capacity in young pigs: effects of age and weaning system. J. Anim. Sci. 55:1380-1387.

Falkowski, J. F., and F. X. Aherne. 1984. Fumaric and citric acid as feed additives in starter pig nutrition. J. Anim. Sci. 58:935-938.

Fan, M. Z., W. C. Sauer, R. T. Hardin, and K. A. Lien. 1994. Determination of apparent ileal amino acid digestibility in pigs: effect of dietary amino acid level. J. Anim. Sci. 72:2851-2859.

FAO. 2014. The Status of fishery resources The State of world fisheries and Aquaculture. FAO, Rome. p 37-40.

FDA. 2017. FACT SHEET: Veterinary Feed Directive Final Rule and Next Steps. https://www.fda.gov/animalveterinary/developmentapprovalprocess/ucm449019.htm Accessed November 3, 2017 2017.

Fell, B. F. 1961. Cell shedding in the epithelium of the intestinal mucosa: Fact and artefact. J. Pathol. Bacteriol. 81:251-254.

Friesen, K. G., R. D. Goodband, J. L. Nelssen, F. Blecha, D. N. Reddy, P. G. Reddy, and L. J. Kats. 1993. The effect of pre- and postweaning exposure to soybean meal on growth performance and on the immune response in the early-weaned pig. J. Anim. Sci. 71:2089-2098.

Giesting, D., and R. Easter. 1985a. Response of starter pigs to supplementation of corn-soybean meal diets with organic acids. J. Anim. Sci. 60:1288-1294.

Giesting, D. W., and R. A. Easter. 1985b. Response of starter pigs to supplementation of corn-soybean meal diets with organic acids. J. Anim. Sci. 60:1288-1294.

Giesting, D. W., M. A. Roos, and R. A. Easter. 1991. Evaluation of the effect of fumaric acid and sodium bicarbonate addition on performance of starter pigs fed diets of different types. J. Anim. Sci. 69:2489-2496.

Hall, G., and T. Byrne. 1989. Effects of age and diet on small intestinal structure and function in gnotobiotic piglets. Res. Vet. Sci. 47:387-392.

Hampson, D. 1986. Alterations in piglet small intestinal structure at weaning. Res. Vet. Sci. 40:32-40.

Hampson, D., and D. Kidder. 1986. Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. Res. Vet. Sci. 40:24-31.

Hardy, R. W., and A. G. Tacon. 2002. Fish meal: historical uses, production trends and future outlook for sustainable supplies. Responsible marine aquaculture:311-325.

Hasan, M. R. 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends. Food. Sci. Tech. 27:120-128.

Hedemann, M. S., and B. B. Jensen. 2004. Variations in enzyme activity in stomach and pancreatic tissue and digesta in piglets around weaning. Archives of animal nutrition 58:47-59.

Henry, R. W., D. W. Pickard, and P. E. Hughes. 1985. Citric acid and fumaric acid as food additives for early-weaned piglets. Anim. Prod. 40:505-509.

Heo, J., F. Opapeju, J. Pluske, J. Kim, D. Hampson, and C. Nyachoti. 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. Journal of animal physiology and animal nutrition 97:207-237.

Herkelman, K. L., G. L. Cromwell, T. S. Stahly, T. W. Pfeiffer, and D. A. Knabe. 1992. Apparent Digestibility of Amino Acids in Raw and Heated Conventional and Low-Trypsin-Inhibitor Soybeans for Pigs. J. Anim. Sci. 70:818-826.

Horn, N., F. Ruch, G. Miller, K. Ajuwon, and O. Adeola. 2014. Impact of acute water and feed deprivation events on growth performance, intestinal characteristics, and serum stress markers in weaned pigs. J. Anim. Sci. 92:4407-4416.

Hötzel, M. J., G. P. P. de Souza, O. A. D. Costa, and L. C. P. Machado Filho. 2011. Disentangling the effects of weaning stressors on piglets' behaviour and feed intake: Changing the housing and social environment. Appl. Anim. Behav. Sci. 135:44-50.

Jacela, J. Y., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. L. Nelssen, D. G. Renter, and S. S. Dritz. 2009. Feed additives for swine: Fact sheets - acidifiers and antibiotics. J. Swine Health Prod. 17:270-275.

Johnson, A., and J. Marchant-Forde. 2009. Welfare of Pigs in the Farrowing Environment. In: J. Marchant-Forde (ed.) The Welfare of Pigs. Animal Welfare. Springer Netherlands No. 7. p 141-188.

Jones, C. K., J. M. DeRouchey, J. L. Nelssen, M. D. Tokach, S. S. Dritz, and R. D. Goodband. 2010. Effects of fermented soybean meal and specialty animal protein sources on nursery pig performance. J. Anim. Sci. 88:1725-1732.

Jørgensen, H., W. C. Sauer, and P. A. Thacker. 1984. Amino acid availabilities in soybean meal, sunflower meal, fish meal and meat and bone meal fed to growing pigs. J. Anim. Sci. 58:926-934.

Kats, L., M. D. Tokach, R. D. Goodband, and J. L. Nelssen. 1992. Influence of weaning weight and growth during the first week postweaning on subsequent pig performance.

Khan, A. R., N. Khazanovich-Bernstein, E. M. Bergmann, and M. N. James. 1999. Structural aspects of activation pathways of aspartic protease zymogens and viral 3C protease precursors. Proceedings of the National Academy of Sciences 96:10968-10975.

Kil, D., L. Piao, H. Long, J. Lim, M. Yun, C. Kong, W. Ju, H. Lee, and Y. Kim. 2006. Effects of organic or inorganic acid supplementation on growth performance, nutrient digestibility and white blood cell counts in weanling pigs. Asian Australasian Journal of Animal Sciences 19:252.

Kim, I. B., and G. L. Allee. 2001. Effect of Carbohydrate Sources in Phase I and Phase II Pig Starter Diets. Asian. Austral. J. Anim. 14:1419-1424.

Ko, K. B., G. D. Kim, D. G. Kang, Y. H. Kim, I. D. Yang, and Y. C. Ryu. 2015. The influences of weaning age and weight on carcass traits and meat quality of pigs. Animal Science Journal 86:428-434.

Konstantinov, S. R., A. A. Awati, B. A. Williams, B. G. Miller, P. Jones, C. R. Stokes, A. D. Akkermans, H. Smidt, and W. M. De Vos. 2006. Post-natal development of the porcine microbiota composition and activities. Environmental Microbiology 8:1191-1199.

Kumar, R. 1992. Anti-nutritional factors, the potential risks of toxicity and methods to alleviate them. Legume trees and other fodder trees as protein source for livestock. FAO Animal Production and Health Paper 102:145-160.

Lallès, J. P. 1993. Soy products as protein sources for preruminant and young pigs. Soy in Animal Nutrition:106-125.

Lampromsuk, P., C. Bunchasak, C. Kaewtapee, S. Sawanon, and T. Poeikhampha. 2012. Effect of supplementing acidifiers and organic zinc in diet on growth performances and gut conditions of pigs. J. Appl. Sci. 12:553-560.

Le Dividich, J., and B. Sève. 2000. Effects of underfeeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. Domest. Anim. Endocrin. 19:63-74.

Leibbrandt, V., R. Ewan, V. Speer, and D. R. Zimmerman. 1975. Effect of weaning and age at weaning on baby pig performance. J. Anim. Sci. 40:1077-1080.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. D. Klemm, and R. D. Goodband. 1991. Innterrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. J. Anim. Sci. 69:4062-4069.

Li, Z., G. Yi, J. Yin, P. Sun, D. Li, and C. Knight. 2008. Effects of organic acids on growth performance, gastrointestinal pH, intestinal microbial populations and immune responses of weaned pigs. Asian. Austral. J. Anim. 21:252-261.

Lindemann, M. D., S. G. Cornelius, S. M. El Kandelgy, R. L. Moser, and J. E. Pettigrew. 1986. Effect of age, weaning and diet on digestive enzyme levels in the piglet. J. Anim. Sci. 62:1298-1307.

Lusk, J. L., F. B. Norwood, and J. R. Pruitt. 2006. Consumer demand for a ban on antibiotic drug use in pork production. American Journal of Agricultural Economics 88:1015-1033.

Mackie, R. I., A. Sghir, and H. R. Gaskins. 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. The American journal of clinical nutrition 69:1035s-1045s.

Main, R. G., S. S. Dritz, M. D. Tokach, R. D. Goodband, and J. L. Nelssen. 2005. Effects of weaning age on growing-pig costs and revenue in a multi-site production system. J Swine Health Prod 13:189-197.

Manners, M. 1976. The development of digestive function in the pig. Proceedings of the Nutrition Society 35:49-55.

Martin, P. 1984. The meaning of weaning. Anim. Behav. 32:1257-1259.

McCracken, K., and D. Kelly. 1993. Development of digestive function and nutrition/disease interactions in the weaned pig. Recent advances in animal nutrition in Australia:182-192.

Min, B. J., J. W. Hong, O. S. Kwon, W. B. Lee, Y. C. Kim, I. H. Kim, W. T. Cho, and J. H. Kim. 2004. The effect of feeding processed soy protein on the growth performance and apparent ileal digestibility in weanling pigs. Asian. Austral. J. Anim. 17:1271-1276.

MPB. 2016. Are you ready for January 1, 2017? http://www.mnpork.com/ready-january-1-2017/ Accessed November 5, 2016.

Nagy, B., and P. Z. Fekete. 1998. Enterotoxigenic Escherichia coli (ETEC) in farm animals. Veterinary research 30:259-284.

Newberry, R. C., and J. C. Swanson. 2008. Implications of breaking mother–young social bonds. Appl. Anim. Behav. Sci. 110:3-23.

Newberry, R. C., and D. G. M. Wood-Gush. 1985. The Suckling Behaviour of Domestic Pigs in a Semi-Natural Environment. Behaviour. 95:11-25.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. ed. Natl. Acad. Press, Washington, DC.

Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51:215-236.

Radecki, S. V., M. R. Juhl, and E. R. Miller. 1988. Fumaric and citric acids as feed additives in starter pig diets: effect on performance and nutrient balance. J. Anim. Sci. 66:2598-2605.

Ravindran, V., and E. Kornegay. 1993. Acidification of weaner pig diets: a review. Journal of the Science of Food and Agriculture 62:313-322.

Richert, B. T. 2010. Feed Additives for Swine. In: D. J. Meisinger (ed.) National Swine Nutrition Guide. U.S. Pork Center of Excellence, Ames, IA. p 173-183.

Shannon, M. C., and G. L. Allee. 2010. Protein and Amino Acid Sources for Swine Diets. In: D. J. Meisinger (ed.) National Swine Nutrition Guide. U.S. Pork Center of Excellence, Ames, IA. p 30-36.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed. Sci. Tech. 59:215-221.

Sindelar, S. C. 2014. Utilization of soybean products as fish-meal protein replacements in Yellow Perch Perca flavescens feeds. M.S., South Dakota State University.

Sinn, S., W. Gibbons, M. Brown, J. DeRouchey, and C. Levesque. 2016. Evaluation of microbially enhanced soybean meal as an alternative to fishmeal in weaned pig diets. Animal.:1-10.

Song, Y. S., V. G. Pérez, J. E. Pettigrew, C. Martinez-Villaluenga, and E. G. de Mejia. 2010. Fermentation of soybean meal and its inclusion in diets for newly weaned pigs reduced diarrhea and measures of immunoreactivity in the plasma. Anim. Feed. Sci. Tech. 159:41-49.

Stangel, G., and P. Jensen. 1991. Behaviour of semi-naturally kept sows and piglets (except suckling) suring 10 days postpuartum. Appl. Anim. Behav. Sci. 31:211-227.

Stein, H. H. 2002. Experience of feeding pigs without antibiotics: a European perspective. Animal biotechnology 13:85-95.

Stein, H. H., B. Seve, M. F. Fuller, P. J. Moughan, and C. F. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180.

Stoner, G. R., G. L. Allee, J. L. Nelssen, M. E. Johnston, and R. D. Goodband. 1990. Effect of select menhaden fish meal in starter diets for pigs. J. Anim. Sci. 68:2729-2735.

Tokach, M., J. Nelssen, and G. Allee. 1989. Effect of protein and (or) carbohydrate fractions of dried whey on performance and nutrient digestibility of early weaned pigs. J. Anim. Sci. 67:1307-1312.

Tsiloyiannis, V. K., S. C. Kyriakis, J. Vlemmas, and K. Sarris. 2001. The effect of organic acids on the control of porcine post-weaning diarrhoea. Res. Vet. Sci. 70:287-293.

van Beers-Schreurs, H., and E. Bruininx. 2002. Nutritional management to prevent disorders in post-weaning pig health. Nutrition and health of the gastrointestinal tract (ed. MC Blok, HA Vahl, L de Lange, AE van de Braak, G Hemke and M Hessing):135-158.

van der Meulen, J., S. J. Koopmans, R. A. Dekker, and A. Hoogendoom. 2010. Increasing weaning age of piglets from 4 to 7 weeks reduces stress, increases post-weaning feed intake but does not improve intestinal funtionality. Animal. 4:1653-1661.

Viljoen, J., S. E. Coetzeel, J. C. Fick, F. K. Siebrits, and J. P. Hayes. 2000. Evaluation of in vivo techniques for the determination of apparent ileal amino acid digestibilities in feedstuffs for piglets. S. Afr. J. Anim. Sci. 30:7-15.

Walsh, M. C., D. M. Sholly, R. B. Hinson, S. A. Trapp, A. L. Sutton, J. S. Radcliffe, J. W. Smith II, and B. T. Richert. 2007. Effects of Acid LAC and Kem-Gest acid blends on growth performance and microbial shedding in weanling pigs. J. Anim. Sci. 85:459-467.

Wang, J. P., S. M. Hong, L. Yan, J. H. Cho, H. S. Lee, and I. H. Kim. 2011. The evaluation of soybean meals from 3 major soybean-producing countries on productive performance and feeding value of pig diets1. J. Anim. Sci. 89:2768-2773.

Weary, D. M., J. Jasper, and M. J. Hötzel. 2008. Understanding weaning distress. Appl. Anim. Behav. Sci. 110:24-41.

Yun, J., I. Kwon, J. Lohakare, J. Choi, J. Yong, J. Zheng, W. Cho, and B. Chae. 2005. Comparative efficacy of plant and animal protein sources on the growth performance, nutrient digestibility, morphology and caecal microbiology of early-weaned pigs. Asian-Aust. J. Anim. Sci 18:1285-1293.

Zhang, Y., and R. Xu. 2006. Anatomy and histology of the gastrointestinal tract. The neonatal pig: Gastrointestinal physiology and nutrition.

Zhu, C. L., M. Rademacher, and C. F. M. d. Lange. 2005. Increasing dietary pectin level reduces utilization of digestible threonine intake, but not lysine intake, for body protein deposition in growing pigs. J. Anim. Sci. 83:1044-1053.